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**ANTENATAL DEPRESSION AND DEVELOPMENTAL PROGRAMMING OF OFFSPRING
HYPOTHALAMICPITUITARY
ADRENAL AXIS IN THE FIRST YEAR OF LIFE**

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**ANTENATAL DEPRESSION AND DEVELOPMENTAL
PROGRAMMING OF OFFSPRING HYPOTHALAMIC-
PITUITARY-ADRENAL AXIS IN THE FIRST YEAR OF
LIFE**

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**Thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy**

Institute of Psychiatry, Psychology and Neuroscience
King's College London

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Dedicated to my children Niamh, Emma and Joe, and to my husband David, with love.

Abstract

Background: Research in humans demonstrates a link between an adverse environment during development and unfavourable health outcomes in adulthood. Animal research used to understand this phenomenon reveals that exposure to prenatal stress and to glucocorticoids may programme offspring hypothalamic-pituitary-adrenal (HPA) axis, which is in turn hypothesised to mediate disease risk. Extension of these findings in human research is at a less advanced stage, although synthesis of a number of lines of evidence suggests that similar HPA axis programming exists. Glucocorticoids are hypothesised as the final mediator in the pathway from adverse antenatal environment to programming effects, including altered offspring HPA axis activity, although molecular mechanisms underlying this hypothesis are yet to be elucidated. Depression in pregnancy is a paradigm by which mechanisms for developmental programming may be further studied.

Methodology: A prospective longitudinal observational study of 82 pregnant women from the second trimester of pregnancy, and their offspring to 1 year postnatal is described. A cases group with DSM-IV major depressive disorder (MDD) was compared with a non-depressed control group. Maternal mood, antenatal HPA axis, obstetric outcomes and infant basal HPA axis activity and cortisol response to pain stress were assessed.

Results: Compared with the control group, women with MDD in pregnancy had overactivity of the HPA axis in the third trimester of pregnancy, a shorter length of gestation, infants with larger cortisol response to pain stress at 8 weeks and 1 year postnatal and higher evening cortisol at 1 year of age. Furthermore, associations were found between antenatal depression, maternal antenatal HPA axis and infant HPA axis. The findings were independent of socio-demographic and obstetric factors and maternal postnatal mood.

Conclusions: The associations between maternal antenatal depression and altered maternal and infant HPA axis activity suggest programming effects and add to the important literature on developmental programming in humans. Furthermore, the findings have clinical relevance in the fields of obstetrics, psychiatry and paediatrics.

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List of abbreviations

11 β -HSD-2	11 β -hydroxysteroid dehydrogenase
ACTH	Adrenocorticotrophic hormone
ALSPAC	Avon Longitudinal Study of Parents and Children
AND	Antenatal depression
AUC	Area under the curve
AUCg	Area under the curve with respect to zero (ground)
AUCi	Area under the curve with respect to awakening level (increase)
Bayley	Bayley scales of infant and toddler development
BDI	Beck Depression Inventory
BME	Black and minority ethnic
BMI	Body mass index
CAR	Cortisol awakening response
CES-D	Center for Epidemiologic Studies Depression Scale
CI	Confidence interval
CORT	Corticosterone
CRH	Corticotrophin-releasing hormone
CRHBP	Corticotrophin-releasing hormone binding protein
DSM	Diagnostic and Statistical Manual
EDD	Expected date of delivery of Mental Disorders
ELISA	Enzyme-linked immunosorbent assay
EPDS	Edinburgh Postnatal Depression Scale
GR	Glucocorticoid receptor
HPA	Hypothalamic-pituitary-adrenal
ICD	International Classification of Diseases
IoP	Institute of Psychiatry
IUGR	Intrauterine growth restriction
KCH	King's College Hospital
KCL	King's College London
LBW	Low birth weight

M	Mean
MDD	Major depressive disorder
MR	Mineralocorticoid receptor
NBAS	Neonatal Behavioural Assessment Scale
NCS-R	National Comorbidity Surveys Replication
OR	Odds ratio
PND	Postnatal depression
PRN	<i>Pro re nata</i> (as required (medication))
PTB	Preterm birth
RR	Relative risk
SCID-I	Structured Clinical Interview for DSM-IV Axis I Disorders
SD	Standard deviation
SE	Standard error of the mean
SGA	Small for gestational age
SIDS	Sudden infant death syndrome
STAI	State Trait Anxiety Inventory
STAIS	State Trait Anxiety Inventory, state score
UTI	Urinary tract infection
WHO	World Health Organisation
WTAR	Wechsler Test of Adult Reading

Chapter 1 INTRODUCTION

1.1 Overview

The broad theme of this thesis is developmental programming, the concept that the environment of the developing organism shapes its development. In this context I describe a study of major depressive disorder in pregnancy, the associated biology in the principal endocrine system regulating the body's response to stress, the hypothalamic-pituitary adrenal (HPA) axis, the obstetric outcomes and the offspring HPA axis. An understanding of these discrete topics provides the necessary background for their synthesis into the single overall framework of this study and is key to the complex theme of developmental programming, as it pertains to antenatal depression in particular.

Accordingly, the introduction provides a description of each of these areas in turn. I start by reviewing the clinical, epidemiological and biological features of major depression in section 1.2.2 before focusing on depression in pregnancy in section 1.2.4. Next I have reviewed normal pregnancy and its biology in section 1.3.2 before describing the epidemiology, biological features and consequences of the major adverse obstetric outcomes in sections 1.3.3 to 1.3.5. Subsequently, since I have assessed the HPA axis in pregnancy and in the infant, I first review the HPA axis in general in section 1.4.2; next I focus on the HPA axis in major depression in section 1.4.3, then the HPA axis in pregnancy in section 1.4.4 and lastly the HPA axis in antenatal depression in section 1.4.5. In the final section I have reviewed developmental programming, firstly focusing on studies in animals including research paradigms, outcomes and mechanisms in section 1.5.2. Next I have reviewed developmental programming studies in humans in section 1.5.3, beginning with the effects of prenatal stress in general, next the specific effects of antenatal depression and finally a review of proposed mechanisms. Lastly, in section 1.6, I present my hypotheses in the context of the topics reviewed in the introduction.

1.2 Depression

1.2.1 Introduction

The lifetime prevalence of major depressive disorder is highest in women, particularly during the childbearing years (Kessler et al., 2010); accordingly, its occurrence during the perinatal period (pregnancy and the first postnatal year) is not unexpected. Historically, there has been much focus on postnatal depression, both in the medical literature and the broader media; correspondingly much research effort has been directed towards postnatal depression. Despite its recognition as early as the 19th century, as indicated by the recording in the psychiatric literature of several series of case reports of 'prepartum melancholia' (Brockington, 1996), the relatively recent recognition of the importance of major depression occurring *during* pregnancy is now reflected by an increase in the volume of research pertinent to the antenatal period. The absence of 'antenatal depression' in the scientific and public consciousness may have been related to unsupported cultural beliefs that pregnancy is a period of happiness and fulfilment for women, which thus protects against depression.

The effect of antenatal depression on pregnancy and on the maternal and offspring biological stress system is the principal focus of this thesis. Thus, depression, and particularly its occurrence in relation to pregnancy, is described in sections 1.2.2 to 1.2.4 below. I start by reviewing the clinical, epidemiological and biological features of major depression in section 1.2.2. Next I include a section on postnatal depression, its pertinence relates to the fact that antenatal depression is likely to have been an unmeasured confounding factor in much research of postnatal depression (see section 1.2.3 below), thus an understanding of postnatal depression will inform that of antenatal depression which is discussed in section 1.2.4. In addition to reviewing clinical, epidemiological and biological features of perinatal depression, within the respective sections I have included a review of the consequences of perinatal depression in order to emphasize the importance and potential impact of depression in the perinatal period.

1.2.2 Major Depressive Disorder

Depression is a common mental disorder; the World Health Organisation has ranked it as the 4th leading cause of disability worldwide, and predicted it to be the leading cause by 2030, demonstrating that it is a significant public health issue (Mathers et al., 2008). Major depression is

associated with burden in many domains including disruption in personal and family relationships (Breslau et al., 2011, Lovejoy et al., 2000), unemployment (Kawakami et al., 2012) and increased morbidity and mortality, reviewed by Kessler (Kessler, 2012); the associated financial costs are high therefore. Depression has been given a variety of apposite epithets by some well-known sufferers. The following examples convey the negative and pervasive nature of this disorder: 'Black Dog', used by the 18th century literary figure, Samuel Johnson, and later by the British Prime Minister Sir Winston Churchill, 'a dark destroyer' by developmental biologist Professor Lewis Wolpert and 'Darkness Visible' by novelist William Styron.

1.2.2.1 Classification and clinical features

Depression is characterised by the core symptoms of low mood and/or anhedonia and a combination of other symptoms including the biological symptoms (fatigue or lack of energy, psychomotor agitation or retardation, sleep disturbance, appetite disturbance) and the cognitive symptoms (poor concentration, worthlessness, guilt and suicidal thoughts or actions). The two major systems of disease classification are broadly similar for depression, which is listed within mood disorders; the term 'major depressive disorder' is used in Diagnostic and Statistical Manual (DSM-5) (APA, 2013) and 'depressive episode' in the International Classification of Disease (ICD-10) (World Health Organization., 1992). Operationally defined criteria of the symptoms listed above must be met in order to make a diagnosis; in DSM-5, duration of symptoms is at least 2 weeks with at least 5 symptoms that must cause clinically significant distress or impairment. Depression may be further classified by severity into mild, moderate or severe with or without psychotic symptoms. The pattern of unipolar depression ranges from a single episode, a remitting and relapsing course to chronic depression.

1.2.2.2 Epidemiology

A recent large-scale epidemiological study of major depression in over nine thousand adults in the USA, The National Comorbidity Surveys Replication (NCS-R), estimated a lifetime prevalence of a major depressive episode of 19.2% and a 12-month prevalence of 8.3% (Kessler et al., 2010). Another well-designed retrospective study has provided similar prevalence data (Wells et al., 2006). However, when studied prospectively, prevalence is much higher: lifetime prevalence in the age group 18-32 years was approximately double, at 41% (Moffitt et al., 2010). Depression is more prevalent in women of all age groups; findings from the NCS-R estimated that the lifetime

prevalence in women of childbearing age was 25.2% compared to 16.8% in men of the same age range (Kessler et al., 2010). Major depression is additionally associated with a number of socio-demographic factors including unemployment, the divorced or never married, less than 12 years of education and poverty (Kessler et al., 2003). Prevalence is higher in high-income countries (Weissman et al., 1996, Andrade et al., 2003, Bromet et al., 2011). Psychiatric comorbidity is common, particularly anxiety and substance use disorders (Kessler et al., 2003). The mean age of onset falls between 24.8 and 34.8 years across 10 countries worldwide (Weissman et al., 1996). Major depression is commonly recurrent over the lifetime; results of a prospective study in the USA suggested that 54% of those with an episode of major depression were likely to recover within 6 months, 70% by 1 year and 81% by 2 years; the remaining 19% continued to be symptomatic for at least 5 years. At fifteen-year follow-up of the same cohort, relapse had occurred in 85% of those who had remitted following the initial episode. Only 11% never experienced a further episode following remission (Keller et al., 1992, Mueller et al., 1999).

1.2.2.3 Aetiology

The aetiology of depression is complex and is not fully understood, although evidence indicates that both genetics and stress play important roles. However, despite the fact that the heritability of unipolar depression is in the region of 40% (Sullivan et al., 2000), in the main, genome-wide association studies have not identified genes for depression (Wray et al., 2012), although one study of a phenotypically homogeneous sample has identified two loci on chromosome 10 which contribute to risk of MDD (Cai et al., 2015). Stress, both early-life and recent, has an aetiological role (Mazure, 1998); however there is inter-individual vulnerability to its propensity to culminate in depression. An expanding body of research since 2003, reviewed by Keers et al., suggests that the effects of environment on the individual may vary according to that individual's genotype (Keers and Uher, 2012). A number of pathophysiological mechanisms for the development of depression have been suggested, including over activity of the hypothalamic-pituitary-adrenal (HPA) axis (Nemeroff, 1996) and of inflammatory responses (Howren et al., 2009), reduced brain neurotrophins (Autry and Monteggia, 2012) and reduced noradrenergic and serotonergic neurotransmission. There are also associated structural (Videbech and Ravnkilde, 2004, Campbell et al., 2004) and functional brain changes (Drevets, 1998).

1.2.2.4 Treatment

The mainstay of treatment of major depression is pharmacological. The main classes of antidepressant drugs, developed since the 1950s, are tricyclic, monoamine oxidase inhibitor, selective serotonin reuptake inhibitor and serotonin noradrenalin reuptake inhibitor. Mood stabilizing drugs are used in recurrent or resistant depression (e.g. lithium or sodium valproate). Other examples of physical treatments are electroconvulsive therapy (ECT) and transcranial magnetic stimulation. Psychological therapies, particularly cognitive behavioural therapy are also used; good practice dictates that attention is paid to psychological and social factors in the management of depression (Taylor et al., 2012).

1.2.3 Postnatal depression

1.2.3.1 Introduction

Major depressive disorder (MDD) occurring in the postnatal period is common and can have serious and long lasting consequences for the mother, her infant and family. It seems that postnatal depression (PND) is simply an episode of MDD that occurs post-natally, and as discussed in section 1.2.1 above, this is not unexpected since the prevalence of MDD is highest in women during their childbearing years. Brockington, a pre-eminent figure in perinatal psychiatry research, suggested that the main strengths of the construct of PND are to highlight it as a public health issue, reduce stigma and enable women to present to services and obtain treatment (Brockington, 1996). As the majority of research in perinatal mental health has, historically, tended to focus on postnatal rather than antenatal depression, it is worthy of comment that in one prospective study of 360 women, 50% of cases of “postnatal depression” began in the antenatal period and approximately one third of the cases of antenatal depression continued to be depressed in the postpartum period (Gotlib et al., 1989). Thus antenatal depression is likely to have been an unmeasured confounding factor that may account for some findings from research of postnatal depression; it therefore warrants more detailed description in sections 1.2.3.2 to 1.2.3.6 below.

1.2.3.2 Classification and clinical features

The clinical symptoms of PND are indistinguishable from MDD outside of the perinatal period (O'Hara and McCabe, 2013). ICD-10 does not specify PND, although it is possible to code for disorders associated with the puerperium with an onset within 6 weeks of delivery. DSM-IV

includes a specifier for MDD 'with postpartum onset' occurring within 4 weeks of parturition. In the preparation of DSM-5 it was initially proposed to increase this window to within 6 months of parturition, however, the final decision, leading to a degree of controversy, was instead a 'peripartum onset' specifier which includes pregnancy and the first 4 postnatal weeks (APA, 2013). These differences and deliberations illustrate the artificial time frame assigned to the construct of PND.

1.2.3.3 Epidemiology

Epidemiological studies of PND provide a range of results, due to differences in the time frame measured (as discussed in section 1.2.3.2 above), whether diagnostic interviews or self-report questionnaires were used, and the population studied. A systematic review of 109 studies performed in developed countries using a diagnostic interview, suggested a period prevalence of depression in the first 3 months postpartum was 7.1% and the incidence of a new episode of depression during the same period was 6.5%. One conclusion of this paper was that a significant amount of uncertainty persists (Gavin et al., 2005). The prevalence of PND in developing countries is likely higher, in the region of 20% (Fisher et al., 2012). Risk factors for PND, reviewed by O'Hara et al., include past history of depression, depression and anxiety during the index pregnancy, neuroticism, low self-esteem, postpartum blues, stressful life-events, poor marital relationship, poor social support, low socioeconomic status, being single, an unwanted pregnancy and difficult infant temperament (O'Hara and McCabe, 2013).

1.2.3.4 Aetiology

Since there is little evidence to support PND as a distinct clinical entity, the aetiology should not differ from MDD in general. None-the-less, there may be a subset of women whose depression is specifically linked to childbirth (Cooper and Murray, 1995), whereas in others with PND, childbirth probably represents a general stressor in women already predisposed to depression. Some studies have shown that certain women may be vulnerable to PND by their sensitivity to changes in the levels of the gonadotrophic hormones, oestradiol and progesterone, as occurs at the time of childbirth or that gonadotrophins produce down-stream effects by their modulation of serotonin and dopamine neurotransmitter systems (Bloch et al., 2000, Bloch et al., 2003, Bloch et al., 2005). Dysregulation of the HPA axis is another postulated mechanism in a subgroup of women with PND (Workman et al., 2012, Meltzer-Brody, 2011, Magiakou et al., 1996b), furthermore there

is complex interaction between the HPA axis and gonadotrophins. Although there appears to be some familial element to PND (Forty et al., 2006), the role of genes in the aetiology of PND remains to be elucidated.

1.2.3.5 Treatment

The treatment of PND is as for MDD occurring outside of the postnatal period, however it is complicated by exposure of the infant to psychotropic medications that are excreted in breast milk. Furthermore, the practicalities of childcare and breastfeeding may be a barrier to treatment with psychological therapies in the postpartum. Overall, the infant and wider family are important considerations when planning the management of PND, and appropriate care of the infant must be ensured.

1.2.3.6 Outcome

The outcome of PND differs from MDD occurring at other times because the sufferer is generally the main caregiver for the new infant, thus it impacts not just on the mother, but also the infant.

1.2.3.6.1 The sufferer

An episode of PND may be of long duration; furthermore the majority of women who experience an episode of PND are likely to experience subsequent episodes of MDD during their child's lifetime (Hay et al., 2008); this potentially results in cumulative exposure of the infant to maternal negative affect over childhood. Moreover, one grave outcome of PND, maternal suicide, was the leading cause, accounting for 28%, of indirect maternal deaths in the UK 1997-1999 (Oates, 2003).

1.2.3.6.2 The offspring

Much research has focussed on the effect of maternal depression, and more specifically PND, on the offspring. There is a clear association with behavioural and emotional problems during childhood (Murray et al., 2011, Avan et al., 2010). Cognitive development is adversely effected, with exposure to PND predicting poorer IQ and language development (Grace et al., 2003, Sohr-Preston and Scaramella, 2006, Brand and Brennan, 2009); the effect being stronger in boys (Brand and Brennan, 2009, Grace et al., 2003). The poor childcare practices discussed below may affect offspring physical health. Furthermore, It is likely that the severity and chronicity of

maternal depression also affects these outcomes (Brennan et al., 2000, Sohr-Preston and Scaramella, 2006). Although research on mediating factors is needed, it is clear that PND is associated with poor childcare practices, for example, poor attendance at child health visits, decreased immunization uptake, poor adherence to safe infant sleeping positions, general safety precautions (Field, 2010, Conroy et al., 2010), an increased potential for child abuse and neglect (Cadzow et al., 1999, Pawlby et al., 2011) and impaired or negative mother-infant interactions (Field, 2010).

1.2.4 Antenatal depression

1.2.4.1 Introduction

Just as at other times in women's lives, MDD may occur during pregnancy, either as a continuation of an episode during which conception occurred, a discrete episode beginning in pregnancy in a woman with a history of recurrent MDD, or a first episode of depressive illness. Significantly, in addition to those of MDD occurring outside of pregnancy, the consequences of antenatal depression (AND) include the effect on the pregnancy, foetus and offspring; since this is a principal focus of the thesis, antenatal depression is described in detail in sections 1.2.4.2 to 1.2.4.6 below.

1.2.4.2 Classification and clinical features

ICD-10 and DSM-IV do not specify antenatal depression, although, as described in section 1.2.3.2 above, the recently adopted DSM-5 now includes a 'with peripartum onset' specifier. The clinical features of antenatal depression do not differ from MDD at other times (Nylen et al., 2013). It has been suggested that some symptoms of depression might be confused with 'symptoms' of pregnancy, for example, tiredness and fatigability, sleep and appetite disturbance (Yonkers et al., 2011, Bloch et al., 2000); however O'Hara reported that these symptoms remain good indicators of mood disorder in the post partum despite their common occurrence in women without mood disorder (O'Hara and McCabe, 2013); it would seem likely, therefore, that the same would apply to the antenatal period when these types of somatic 'symptoms' also occur in non-depressed women.

1.2.4.3 Epidemiology

A systematic review of prevalence and incidence of perinatal depression in developed countries identified 12 studies that used diagnostic interviews in pregnancy; the incidence of a new episode of MDD occurring during pregnancy was 7.5%. There were few studies of period prevalence; however, the best estimate of MDD occurring during pregnancy was 12.7% (Gavin et al., 2005). Higher prevalence rates, between 18 and 39%, are reported in epidemiological studies in developing countries (Rahman et al., 2003, Karmaliani et al., 2009, Kazi et al., 2006). A systematic review of risk factors for depressive symptoms during pregnancy identified maternal anxiety, life stress, personal history of depression, lack of social support, domestic violence, unintended pregnancy, low income, lower education, smoking and relationship factors (Lancaster et al., 2010). Compared with women who have not experienced childhood maltreatment, those who have, are ten times more likely to experience depression in pregnancy (Plant et al., 2013).

1.2.4.4 Aetiology

Although the aetiology of antenatal depression has received little research attention, one would expect it to mirror that of MDD at any other time (see section 1.2.2.3 above); however, pregnancy presents a unique period of altered biology, which may also play a role in the aetiology of MDD occurring at this particular time.

1.2.4.4.1 HPA axis

Section 1.2.2.3 above describes overactivity of the HPA axis as a proposed pathophysiological mechanism in MDD outside of pregnancy. This is one of the most consistent biological findings in psychiatry (Pariante and Lightman, 2008). Furthermore, normal pregnancy heralds a profound change in the HPA axis as the placenta takes over secretion of corticotrophin-releasing hormone (CRH), which is produced in exponentially increasing amounts to term (McLean et al., 1995). Taken together, these facts point to the HPA axis as a potential candidate in understanding the aetiology of antenatal depression, although this line of research is still in its infancy. The HPA axis and MDD in pregnancy are one focus of this thesis therefore this topic is described in detail in section 1.4.5 below. The HPA axis as a proposed aetiological factor in antenatal depression has been most studied; however, other systems are described below for completeness.

1.2.4.4.2 Immune system

Inflammatory responses are a proposed pathophysiological mechanism in MDD outside of pregnancy (see section 1.2.2.3 above). Intriguingly, pregnancy is a period of altered inflammatory and immune system responses (Sykes et al., 2012); furthermore, inflammation is associated with some pregnancy-related disorders such as preeclampsia and preterm birth, which are, in turn, associated with antenatal depression (Osborne and Monk, 2013). There are few studies of inflammation and depression in pregnancy but they suggest a possible role of the immune system. Although research in this field is in its infancy, antenatal depression has been associated with interleukins 1 β and 6 (IL-1 β , IL-6) (Cassidy-Bushrow et al., 2012, Christian et al., 2009), C-reactive protein (CRP) (Scrandis et al., 2008), macrophage migration inhibitory factor (MIF) (Christian et al., 2010) and tumour necrosis factor alpha (TNF α) (Haeri et al., 2013).

1.2.4.4.3 Sex hormones

The levels of oestrogen and progesterone increase throughout gestation, however little data is available for any role in antenatal depression. O'Keane et al. found no statistically significant difference in progesterone between depressed and healthy pregnant women at 25 or 36 weeks gestation (O'Keane et al., 2011). None-the-less, the research cited in section 1.2.3.4 above suggests that changes in levels of sex hormones may play an aetiological role in the development of PND (Bloch et al., 2003), thus the same potentially applies to antenatal depression.

1.2.4.4.4 Neurotransmitter systems

As described in section 1.2.3.4 there is interplay between gonadotrophic hormones and serotonergic and other neurotransmitter systems implicated in the aetiology of MDD (see section 1.2.2.3), which again may be relevant to antenatal depression.

1.2.4.4.5 Thyroid function

There is a well-established association between abnormality of thyroid function and depression. Furthermore, physiological changes to the thyroid gland occur in normal pregnancy, thyroid disorders are common in pregnancy and are associated with both maternal and foetal complications (Cignini et al., 2012). Although more research has been performed for PND, an association has been reported between depressive symptoms during pregnancy and total and free thyroxine (Bunevicius et al., 2009, Pedersen et al., 2007).

1.2.4.4.6 Melatonin

Disruption of circadian rhythms is found in MDD and is hypothesized in the aetiology of mood disorders (reviewed by (Germain and Kupfer, 2008). Melatonin is a measure of circadian rhythmicity in humans (Lanfume et al., 2013), however, little research exists on the role of melatonin in antenatal depression. One small study found lower melatonin levels, and a lack of the normal increase in melatonin secretion over the duration of the pregnancy in women with antenatal depression compared with healthy controls (Parry et al., 2008).

1.2.4.4.7 Genetics

A family study has demonstrated a familial element to perinatal depression (MDD in pregnancy and/or postpartum), although the study does not specifically explore antenatal depression. However, the odds ratio for prediction of sibling status for perinatal depression was 2.28 (Murphy-Eberenz et al., 2006). In a small genetic study of 89 pregnant women, symptoms of depression were correlated with the low-activity variants of the genes coding for mono-amine-oxidase type A and catechol-o-methyl-transferase (COMT) and the high-activity variant of the serotonin transporter (5-HTT) (Doornbos et al., 2009), providing preliminary evidence for the role of genes in the aetiology of antenatal depression.

1.2.4.5 Treatment

The management of antenatal depression differs from that for MDD, described in section 1.2.2.4 above, only because consideration must be given to the unborn child, and looking ahead to breastfeeding and the health and wellbeing of the infant in the longer term. The use of antidepressant drugs in pregnancy should be based on a careful risk-benefit analysis and shared decision-making between the doctor and pregnant woman. Electroconvulsive therapy may be used and there is no evidence of harm to the mother or foetus (Miller, 1994). In the UK, the National Institute for Health and Care Excellence has issued guidelines for clinical management of antenatal and postnatal mental health (NICE, 2014).

1.2.4.6 Outcome

The outcome of antenatal depression differs from MDD occurring at other times in that it impacts not just on the mother, but also the pregnancy and unborn child.

1.2.4.6.1 The sufferer

As described for PND (section 1.2.3.6.1 above) and MDD in general, since depression is often a chronic and relapsing disorder, further episodes are likely to be experienced throughout the lifetime. Moreover, one notable outcome is suicide which was the leading cause, accounting for 28%, of all indirect maternal deaths in the UK 1997-1999 (Oates, 2003). Despite this fact, there is a dearth of research into antenatal depression and suicide in pregnancy. Suicidal ideation in the perinatal period has been assessed using the suicidality item of self-report depression rating scales, for example the Edinburgh Postnatal Depression Scale (EPDS (Cox et al., 1987)). Accordingly, suicidal ideation in pregnancy has been found to be relatively prevalent, ranging from 5% and 14% (Lindahl et al., 2005). In women with a diagnosis of major or minor depression in pregnancy the range was much higher at 26%-34% (Mauri et al., 2012). However, the act of suicide in pregnancy is less common than in non-pregnant women of childbearing age. One study found lower rates of suicidality (rated by the Hamilton Depression Rating Scale, (Hamilton, 1960)) in depressed women who were pregnant compared with depressed non-pregnant women (Manber et al., 2008). Furthermore, Appleby found that suicide in pregnancy (all women) was rare (standardized mortality ratio, 0.05) and postulated a protective effect of pregnancy and motherhood (Appleby, 1991).

1.2.4.6.2 The pregnancy

Antenatal depression has been associated with the adverse obstetric outcomes of preterm birth (PTB) (<37 weeks gestation), low birth weight (LBW) (<2500g) and intrauterine growth restriction (IUGR) (<10th percentile for gestational age). Worldwide, these birth outcomes are the leading causes of neurodevelopmental impairments and disabilities and of neonatal and childhood morbidity and mortality (WHO, 1995, Swamy et al., 2008, Allen and Jones, 1986, Wilson-Costello et al., 2005). Two meta-analyses (sharing some of the same data) have examined the relationship between antenatal depression, PTB and LBW (Grigoriadis et al., 2013, Grote et al., 2010); both describe modest but statistically significant risks between antenatal depression and both outcomes, for example, PTB: RR = 1.39; 95% CI, 1.19 to 1.61, and LBW: RR = 1.49; 95% CI, 1.25 to 1.77. One of these meta-analyses also examined IUGR and likewise describes an association with antenatal depression: RR = 1.45; 95% CI, 1.05 to 2.02 (Grote et al., 2010). The associations were strongest in studies of categorically defined depression. The public health

significance was highlighted, as whilst the sizes of the relative risks are modest, antenatal depression is common; the magnitude of the risk of PTB associated with antenatal depression was likened to that of smoking 10 cigarettes per day in pregnancy (Grote et al., 2010). Methodological weaknesses of the studies included in these 2 meta-analyses were noted as important limitations. For example, a number of studies did not control for potential confounding factors such as smoking or antidepressant use; the authors concluded that more research with improved methodologies is required. Indeed only a minority of the studies included in these meta-analyses were of operationally defined MDD as opposed to self-rated symptom levels (Diego et al., 2009, Field et al., 2010, Field et al., 2006a, Suri et al., 2007, Wisner et al., 2009, Rahman et al., 2004). The findings of the two meta-analyses are corroborated by a more recent population-based cross-sectional study in Finland of over 500,000 pregnant women which found that women with physician-diagnosed ICD-10 depression, identified through specialised healthcare units (n = 4120), had increased odds of preterm birth, LBW and SGA babies (Raisanen et al., 2014).

Two other obstetric outcomes, gestational age at birth and birth weight as continua were included in one of the above meta-analyses but no association between antenatal depression and either of these outcomes was found (Grigoriadis et al., 2013). However, contemporaneous publications not included in the meta-analysis have found shorter length of gestation in antenatal depression. In a large (>4000 subjects) birth cohort study an association of shorter length of gestation (albeit in days) and high levels of depressive symptoms in pregnancy is reported (Van Dijk et al., 2010) and a smaller study (n=54) of operationally defined MDD also found a shorter length of gestation in the depressed group (O'Keane et al., 2011). As discussed in section 1.3.4.3 below, shorter length of gestation even within the 'at-term' range is associated with a variety of sub-optimal outcomes; its association with antenatal depression is therefore noteworthy.

Antenatal depression is further likely to have indirect adverse effects on obstetric outcome since it is associated with other risk factors for adverse pregnancy outcomes including health-related behaviours such as smoking (Kyrklund-Blomberg and Cnattingius, 1998, Ellard et al., 1996), substance misuse (Kelly et al., 2002), alcohol, poor weight gain, poor attendance for antenatal care (Zuckerman et al., 1989), and medical problems such as hypertension (Haelterman et al., 1997, Sibai et al., 2000), pre-eclampsia (Kharaghani et al., 2012, Kurki et al., 2000, Xiong et al., 1999) and diabetes (Kozhimannil et al., 2009, Evers et al., 2004).

Lastly, since women with antenatal depression may require treatment with antidepressants during their pregnancy, the effect of these drugs should also be considered. A recent meta-analysis provided data for pregnant women who received antidepressants during pregnancy and those who did not (either with or without current depression). Antidepressant exposure was associated with lower gestational age (mean difference of 0.45 weeks; 95% CI, 0.25 to 0.64) and with PTB (OR = 1.55, 95% CI, 1.38 to 1.74), including when the comparison group was solely women who were depressed during pregnancy. Antidepressant exposure was not associated with reduced birth weight when the comparison group was solely women depressed during pregnancy; in contrast Apgar scores were lower at 1 and 5 minutes, although still within the accepted normal range. Notably, the authors concluded that all the effect sizes were small thus the clinical significance was questioned. Moreover, the amount of available data for the meta-analysis was small and of variable quality, with relatively few studies including data on important confounding factors such as smoking (Ross et al., 2013).

1.2.4.6.3 The offspring

1.2.4.6.3.1 Foetus

The effect of antenatal depression on the foetus was investigated in 2 small studies of women with high or low levels of depressive symptoms; the findings included higher basal heart rate and slower heart rate response to a vibro-acoustic stimulus, which took longer to return to basal levels in the foetuses of the women with symptoms of depression (Allister et al., 2001, Monk et al., 2004).

1.2.4.6.3.2 Neonates

Effects of antenatal depression on neonates include higher urine cortisol and lower urine dopamine and serotonin levels and frontal EEG asymmetry in 2 studies from the same research group of >70 women with high levels of depressive symptoms in pregnancy (Field et al., 2004a, Diego et al., 2004). In the same study (Field et al., 2004a) and one other from that research group (Diego et al., 2005), neonates exposed to symptoms of depression were examined using the Neonatal Behavioural Assessment Scale (NBAS) (Brazelton, 1995) and showed poorer scores in habituation, orientation and motor items. Another study of 39 women with DSM-IV MDD in pregnancy found that male, but not female, neonates had poorer motor scores and regulation of

states according to the NBAS (Gerardin et al., 2010). Examination of >1000 neonates, by a paediatrician, using the Neurologic and Adaptive Capacity Scale (NACS) (Amiel-Tison et al., 1982), showed that symptoms of depression in pregnancy predicted infants who were difficult to console or cried excessively (Zuckerman et al., 1990). Compared with healthy women, in those with high levels of depressive symptoms in pregnancy, initiation of breast feeding was reduced (OR = 0.68; 95% CI, 0.61-0.76; $p < .001$) (Grigoriadis et al., 2013), therefore their infants were not advantaged by the benefits of breastfeeding (Salone et al., 2013).

1.2.4.6.3.3 Infants and older children

In a study of 247 women, depression symptoms in pregnancy predicted negative reactivity in 2-month-old infants (Davis et al., 2007). Compared with 1-year-old infants of a control group of mothers without antenatal depression or PND ($n=79$), the infants of 39 women with DSM-IV MDD in pregnancy had higher scores on anxiety, activity/impulsiveness and sleep problems (Carter et al., 2003) measured with the Infant-Toddler Social and Emotional Assessment (ITSEA) (Gerardin et al., 2010). Using the prospectively ascertained cohort of the Avon Longitudinal Study of Parents and Children (ALSPAC) (Golding et al., 2001), the development of the 18-month-old children of >9000 women, with either low or high levels of depressive symptoms in pregnancy were compared; a modified version of the Denver Developmental Screening Test (Frankenburg et al., 1992) was used. A high level of symptoms of depression throughout pregnancy was associated with developmental delay in the children (OR 1.34, 95% CI 1.1-1.6) (Deave et al., 2008). In the same cohort, there was increased risk of child attention problems, measured with the strengths and difficulties questionnaire (SDQ) (Goodman, 1999) (OR 1.33, 95% CI 1.19-1.48) (Van Batenburg-Eddes et al., 2012). Compared with three-year-old children not exposed ($n=769$), children exposed to a high level of symptoms of depression in pregnancy ($n=69$) were found to have lower body mass index (BMI) and higher central adiposity (Ertel et al., 2010). Regarding older children, a prospective longitudinal study of 120 pregnant women showed a twofold increase in risk for antisocial outcomes, fourfold risk for violent behaviour (Hay et al., 2010) and almost fivefold risk for depression (Pawlby et al., 2009) in the 16-year-old offspring of women diagnosed with MDD in pregnancy. In the same prospective study, the offspring of women who had themselves experienced childhood maltreatment and subsequently had depression in pregnancy, experienced greater levels of maltreatment by age 11 years (Plant et al., 2013). The ALSPAC study, described above, found that 15-year-old adolescents exposed to high levels of

maternal symptoms of depression (and anxiety) in pregnancy had a blunted cortisol awakening response (CAR), suggesting that prenatal mood has a persisting effect on offspring HPA axis; this finding was independent of potential antenatal and postnatal confounding factors and lends support to the developmental programming hypothesis (O'Donnell et al., 2013) (see section 1.5.3.2.1 below). Further findings from the ALSPAC cohort demonstrated that symptoms of antenatal depression were an independent risk factor for offspring MDD aged 18, with a dose-related effect (Pearson et al., 2013).

In summary, the effects of antenatal depression on the offspring are wide-ranging and significant and include adverse effects on health physiology, neurodevelopment, mental health and behaviour.

1.3 Pregnancy and adverse obstetric outcomes

1.3.1 Introduction

As described in section 1.2.4.6.2 above, there is a clear association between MDD in pregnancy and several adverse pregnancy outcomes. Since obstetric outcome is one focus of this thesis, the following section describes these factors and highlights their pertinence to antenatal depression. First I have reviewed normal pregnancy and its biology in section 1.3.2 before describing the epidemiology, aetiology and consequences of the adverse obstetric outcomes of preterm birth, shortened length of gestation, small for gestational age and low birth weight babies in sections 1.3.3 to 1.3.5.

1.3.2 Normal pregnancy

A normal singleton pregnancy lasts 40 weeks from the first day of the last menstrual period. Term gestation is widely accepted to be that occurring between 37 and 42 weeks from the first day of the last menstrual period. These cut-offs have been devised somewhat arbitrarily by dint of the complications that occur in shorter or longer pregnancies. The designated duration of term pregnancy has been adjusted over the years to the most recent cut-off for PTB (<37 weeks) in the 1970s, and for post-term (>42 weeks), in the 1950s (Fleischman et al., 2010).

A normal weight baby falls between the 10th and 90th centiles of a standard growth chart. Birth weight is determined by length of gestation and foetal growth rate. Low birth weight (<2500g) regardless of gestational age occurs either because of preterm delivery or slow foetal growth rate (IUGR) or a combination of both. Babies with weight falling below the 10th centile for their gestational age are termed 'small for gestational age' and those above the 90th centile are termed 'large for gestational age;' this usually occurs due to a high foetal growth-rate (Dunn, 1985).

Pregnancy is traditionally broken down into 3 stages or 'trimesters' of approximately 3 months each. The 1st trimester includes implantation of the embryo to the endometrial lining of the uterus, formation of the placenta and a period of rapid foetal growth and development; the brain, other organs and limbs begin to form. The first trimester is the period of greatest risk for miscarriage and birth defects. Growth and major organ development continue in the 2nd trimester; of relevance to this thesis, foetal HPA axis activity begins in mid-gestation (Mastorakos and Ilias, 2003). The period of highest foetal weight gain occurs in the 3rd trimester.

Many metabolic and physiological changes occur in the pregnant woman's body in order to support the growth and development of the foetus and to prepare for parturition and lactation. The placenta develops both from foetal cells and maternal uterine tissue and forms a barrier between maternal and foetal blood (which do not mix). The placenta has several functions: (i) it allows delivery of nutrients to, and elimination of waste products from the foetus, and gas exchange between mother and foetus; (ii) it allows the development of passive immunity by the transport across the placenta of maternal immunoglobulin G (IgG) antibodies; (iii) by term, the placenta has become the largest endocrine organ in the body; it secretes oestrogens, progesterone, human chorionic gonadotrophin (hCG), human placental lactogen (hPL), and CRH. Progesterone levels rise throughout pregnancy, and decline towards the end; progesterone facilitates implantation, inhibits uterine smooth muscle contraction and inhibits lactation (Feldt-Rasmussen and Mathiesen, 2011). Levels of oestrogens rise steadily from week 10 to term; oestrogens are involved in controlling other pregnancy hormones and in foetal development. Levels of hCG rise exponentially from implantation, peak at 10 weeks gestation, then decline and plateau for the remainder of the pregnancy. hCG has many key roles in pregnancy including embryo implantation, placental development and uterine and foetal growth (Cole, 2012). Levels of hPL rise linearly throughout gestation to peak at 32-35 weeks gestation; hPL appears to modify maternal metabolism to ensure adequate foetal nutrition (Newbern and Freemark, 2011) (see Figure 1). Levels of CRH rise exponentially throughout pregnancy, peaking at delivery (Sasaki et al., 1987, Frim et al., 1988). Of particular relevance to this thesis, CRH is essential for foetal development and maturation, the maintenance of pregnancy and the timing of delivery (McLean et al., 1995, Zoumakis et al., 2009).

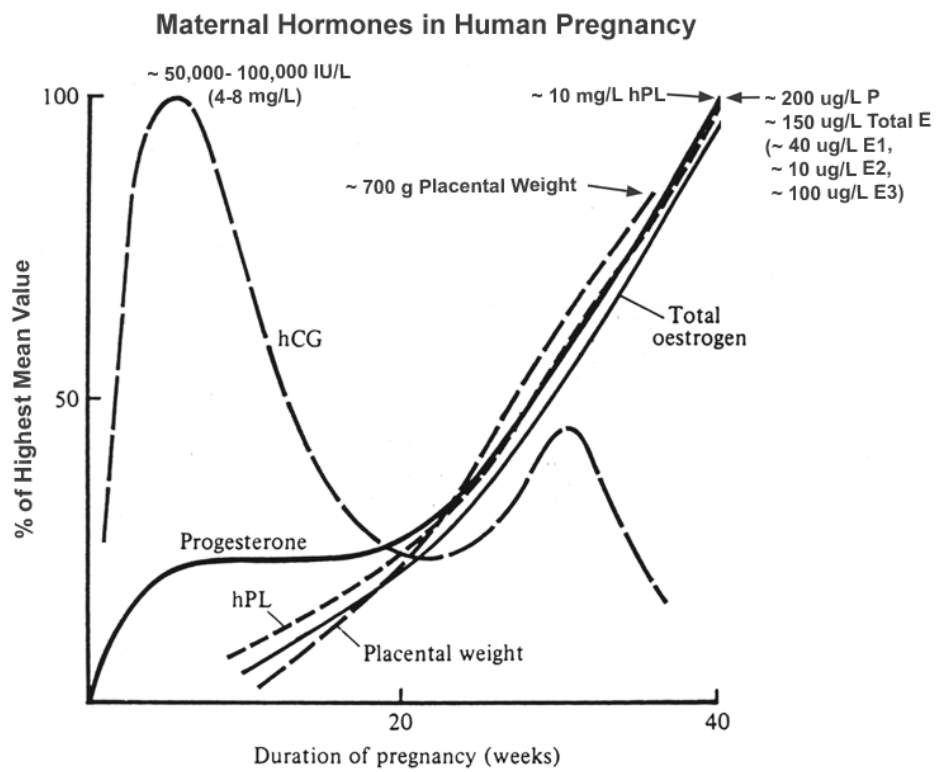


Figure 1: Maternal hormones in pregnancy (Gwatkin, 1989)

1.3.3 Preterm birth

Preterm birth is a major public health concern worldwide; it is associated with significant morbidity and mortality and high costs in health and social care. PTB may be either medically indicated or spontaneous (either spontaneous onset of labour or spontaneous rupture of membranes). The pathways to PTB are complex, and are not yet fully understood.

1.3.3.1 Definition

The World Health Organisation (WHO) defines PTB as less than 37 completed weeks of gestation. Birth weight was originally used as a proxy for prematurity, however this is now considered unsatisfactory as it may miss many preterm babies (Behrman, 2007). Gestational age can be determined using the first day of the last menstrual period but it is commonly confirmed by ultrasound examination, which is known to be accurate, particularly in early pregnancy (Kalish et al., 2004).

1.3.3.2 Epidemiology

The worldwide estimate for PTB in 2010 was between 12.3 and 18.1 million; equivalent to 11.1% of all live births. However, there are marked differences by country, for example rates were 5% in several European countries and 18% in some African countries. PTB also occurs in wealthy countries, for example the USA is one of ten countries worldwide with the highest rates of PTB (Blencowe et al., 2012). Furthermore differences exist within countries, for example in the USA, rates of PTB are 10.9% in white Americans and 17.5% in African-Americans. Worldwide, the majority (82%) are moderate or late PTBs (>32 weeks gestation) where chances of survival and adverse consequences are better than early PTB. PTB is more common with male (55%) than female offspring (Zeitlin et al., 2002). Despite advances in science and healthcare, rates of PTB have been rising over the past 20 years (Blencowe et al., 2012, Goldenberg et al., 2008).

1.3.3.3 Aetiology

The aetiology of PTB is explored in detail in this section since depression and PTB have a number of aetiological factors in common, for example, anxiety, stressful life events, substance misuse, marital status, socio-economic status.

1.3.3.3.1 Psychosocial and stress

Much research has focussed on the relationship between stress and PTB. Historically, research on stress and PTB has been somewhat hampered as depression, anxiety and general distress have not been distinguished. A review in 1999 found that only half of the 20 studies on the theme reported a significant association of stress (including life events, anxiety, depression or emotional distress) with PTB (Savitz and Pastore, 1999). However, in this new century the body of research on stress and PTB has burgeoned, and is strengthened by a number of improvements in methodology. The majority of these more recent investigations have shown significant associations of stress with PTB; it appears that more methodologically rigorous research is providing clearer results (Behrman, 2007). Mechanisms linking stress in pregnancy and PTB are hypothesized to include activation of the HPA axis, alteration in immune function and unfavourable health-behaviours.

1.3.3.3.1.1 Depression

Depression can be conceptualised as a form of chronic stress and as described in detail in section 1.2.4.6.2 above, two recent meta-analyses have supported the finding of increased risk of PTB in antenatal depression (Grote et al., 2010, Grigoriadis et al., 2013).

1.3.3.3.1.2 Anxiety and other types of stress

Although anxiety and depression are often co-morbid they are distinguishable conditions and it is important to disentangle their individual influence on the risk of PTB. The evidence for an effect of general anxiety on PTB is mixed and a meta-analysis of anxiety symptoms in pregnancy showed no significant association with PTB (Littleton et al., 2007). However, it appears that pregnancy-specific anxiety in particular predicts PTB but not an individual's anxiety resulting from knowledge about their own medical risk (Dole et al., 2003, Mancuso et al., 2004, Rini et al., 1999).

Life event and PTB research has provided mixed results, although in general, studies with more rigorous methodology have shown significant results (Behrman, 2007). There are also mixed results for an effect of catastrophic stress occurring during pregnancy on PTB, however, a recent systematic review concluded that there does not seem to be an effect on length of gestation (Harville et al., 2010). Chronic stress in pregnancy, for example, financial, family and work, has been associated with PTB (Misra et al., 2001). A meta-analysis of domestic violence showed an increased risk of PTB (adjusted OR = 1.46; 95% CI, 1.27-1.67) (Shah and Shah, 2010).

1.3.3.3.2 Behavioural

Within this category, cocaine use is the most consistently associated with risk of PTB with an approximate two-fold increase and there is probably a modest association with cigarette smoking, (Berkowitz and Papiernik, 1993).

1.3.3.3.3 Social and demographic

Both young maternal age (Hediger et al., 1997) and maternal age over 35 (Cnattingius et al., 1992) are risk factors for PTB. Single women appear at higher risk for PTB than married women (Raatikainen et al., 2005). Compared with white women, black, but not Asian and Hispanic women are at increased risk of PTB (Schaaf et al., 2012). Several studies in developed countries demonstrate an association between lower socioeconomic status and PTB (Behrman, 2007).

1.3.3.3.4 Medical conditions and pregnancy problems

Chronic and acute medical conditions, previous indicated or spontaneous PTB and family history of PTB all constitute risks. Birth defects and PTB are associated (Rasmussen et al., 2001). A short inter-pregnancy interval (<6 months between the termination of one and conception of another) has been linked to PTB (Smith et al., 2003a). Multiple gestation is associated with PTB, furthermore, assisted reproductive techniques, for example *in vitro* fertilization (IVF), even for singletons have around a two-fold increased risk for PTB (McGovern et al., 2004).

1.3.3.3.5 Genetics

Genetics are thought to play a role in the predisposition to PTB (Dolan, 2010). Twin studies indicate that heritability lies between 20-40% (Treloar et al., 2000, Clausson et al., 2000). The results of a large genome-wide association study (GWAS) are due to be published soon (Dolan and Christiaens, 2013).

1.3.3.3.6 Environmental toxins

Lead, tobacco smoke and air pollution likely contribute to the risk of PTB, but investigation of other environmental toxins is lacking (Behrman, 2007).

1.3.3.3.7 Common biological pathways

As described in the sections above, the causes of PTB are multifactorial and complex and vary according to gestational age; however, there is a common biological pathway to the process of parturition, reviewed by Challis et al., 2000. The HPA axis, a key focus of this thesis, is central to this pathway, which is initiated by HPA axis activation and increased placental CRH expression. This, in turn, leads to a biological cascade including a functional progesterone withdrawal and oestrogen activation, expression and activation of contraction-associated proteins, oxytocin and prostaglandins. This cascade culminates in cervical ripening, uterine contractility and decidual and foetal membrane activation (Challis et al., 2000). PTB occurs when pathological processes activate one or more elements of this pathway before full term. These processes include stress, systemic or genital tract infection, placental ischaemia or vascular lesions and uterine over-distension (Behrman, 2007). PTB is preceded by preterm premature rupture of membranes (PPROM) in 40% of cases; again this is part of the final common pathway to PTB. With regards to HPA axis involvement in PTB, compared with women delivering at term, 2nd trimester plasma CRH has been shown to be significantly higher in those who go on to deliver preterm, and lower in those delivering post-term (McLean et al., 1995). The term 'CRH placental clock' has been used, active from early pregnancy, length of gestation and timing of delivery is thus determined (McLean et al., 1995) and CRH in early third trimester has been shown to predict length of gestation (Wadhwa et al., 1998). Furthermore levels of the binding protein (CRHBP) have been shown to be lower in the second and third trimesters in pregnancies ending in PTB (Hobel et al., 1999) (see section 1.4.4 below).

1.3.3.4 Outcomes

1.3.3.4.1 Short-term outcomes

Those born at the lower limits of viability have the highest mortality rates and levels of prematurity-related morbidity. Prematurity is one of the leading causes of infant mortality worldwide (Liu et al., 2012) and mortality rates increase with decreasing gestational age (CDC, 2003). In 2010 the infant mortality rate for preterm babies was 2.4% of live births in the UK (ONS, 2012). The more premature the infant, the more immature are the organ systems and the less prepared to support extra-uterine life. Morbidity includes lung disease including respiratory distress syndrome, gastrointestinal disorder, compromised immune system, morphological and functional cardiovascular disease, auditory and ophthalmic problems and neurological insult.

Moreover, the aetiology of the PTB and the biological mechanisms of the common pathway to PTB, for example inflammation and cytokine injury, may also influence infant health outcomes (Behrman, 2007).

1.3.3.4.2 Long-term outcomes

1.3.3.4.2.1 The preterm individual and their family

Prematurity may lead to a wide range of neurodevelopmental disabilities, some with long-term effects into adulthood. These disabilities include cerebral palsy, mental retardation, hearing, sight and language impairments, learning disability, attention deficit-hyperactivity disorder (ADHD), minor neuro-motor dysfunction or developmental coordination disorders, behavioural problems and social-emotional difficulties (Behrman, 2007). The majority of studies on foetal origins of adult disease have used birth weight as the marker of early adversity, however more recent research has used PTB. Insulin resistance (Fewtrell et al., 2000, Hofman et al., 2004b) and metabolic abnormalities (Hofman et al., 2004a) have been reported in pre-teen children who were born preterm. The impact on families of having a child born preterm may be multi-faceted and long-term. Reduced psychological wellbeing, family breakdown, reduced employment opportunities and financial impacts are possible negative factors. Conversely, the impact may be positive, for example, bringing families closer together.

1.3.3.4.2.2 Society

In the USA in 2005, the financial burden to society for each child born preterm, over and above costs associated with a child born at full term, were quoted as 51,600 USD (approximately 34,000 GBP) per year. Costs include the infant's medical care, maternal delivery costs, early intervention services, special education and lost household and labour market productivity (Behrman, 2007).

1.3.4 Shortened length of gestation

1.3.4.1 Introduction

As detailed in section 1.3.2 above, the consensus length of gestation for a full term pregnancy is somewhat arbitrary but is based on the increased risk of complications seen in infants born at <37 or >42 weeks gestation. There has been increasing research interest in the complications that attend infants born early within the at-term range as it has become apparent that infants born at 37 and 38 weeks gestation have significantly higher risks of morbidity and mortality (Kramer et al., 2013, Zhang and Kramer, 2009, Fleischman et al., 2010). Evidence is emerging of correlations

between the severity of some sub-optimal outcomes and shorter length of gestation even within the 'at-term' range (see section 1.3.4.3 below). Thus, for certain outcomes, it appears that it may be more appropriate to consider the effect of gestational age as a continuum rather than PTB as 'all or none' (Fleischman et al., 2010).

1.3.4.2 Depression and shortened length of gestation

Since depression during pregnancy may be associated with shorter length of gestation (the evidence is described in section 1.2.4.6.2 above) an outline of the effects of this particular outcome is warranted. Several large studies on outcomes associated with shortened length of gestation, which control for birth weight and other confounders, are detailed in section 1.3.4.3 below.

1.3.4.3 Outcomes

1.3.4.3.1 Respiratory system

Gestational age is a determinant of the degree of pulmonary maturation and the adequacy of surfactant production, which are critical factors in determining respiratory function at birth. A retrospective study of >180,000 live births in one region of the UK from 1988 – 1992 showed that compared with infants born at 39-41 weeks, infants born at 37-38 weeks gestation were at increased risk of respiratory distress and its associated mortality, particularly when labour was not spontaneous. This increased risk was in the order of a 200-fold increase at 37 weeks. Furthermore, the requirement for ventilator support for respiratory distress was 78 times more likely at 38 than at 39-41 weeks gestation (Madar et al., 1999).

1.3.4.3.2 Sudden Infant Death Syndrome (SIDS)

A study in Scotland of >200,000 live births following spontaneous onset of labour at full term, between 1992-1995, showed the risk of SIDS decreased significantly with each additional week of gestation (OR = 0.72; 95% CI, .60-.86). Compared with infants born at 40 weeks, those born at 37 weeks gestation had an increased risk of sudden infant death (adjusted OR = 2.5, 95% CI, 1.2-5.2) (Smith et al., 2003b).

1.3.4.3.3 Brain development

An MRI study of 67 healthy American children aged 6-10 years who had been born at full term and had a stable neonatal course found that longer gestation was associated with neurodevelopmental benefit i.e. increases in grey matter density in some temporal regions (Davis et al., 2011a). This finding demonstrates that even at full term, shorter length of gestation has lasting effects on neurodevelopment. Disruptions to brain development may increase risk, in the longer term, for the development of behavioural (Gozzo et al., 2009), cognitive (Yang et al., 2010) or psychopathological (Janssens et al., 2009, Wright et al., 2000, Raikkonen et al., 2007) problems.

1.3.4.3.4 Cognitive ability

A study of >13,000 6 year old children born at full term and of normal weight in the 1990's in Belarus compared the IQ (using the Wechsler Abbreviated Scales of Intelligence) of children born at 39-41 weeks with those born at 37 and 38 weeks gestation. The full-scale IQ was 1.7 (95% CI - 2.7, -0.7) and 0.4 (95% CI -1.1, 0.02) points lower in children born at 37 and 38 weeks gestation respectively (Yang et al., 2010). A retrospective population-based study, in Scotland, of >17,000 school-aged children showed an increasing risk of special educational need (SEN) with decreasing gestational age at birth which held true both for preterm and full term births. There was increased risk of special educational need for children born at 33-36 weeks (adjusted OR = 1.53; 95% CI, 1.43-1.63) and at 37-39 weeks (OR = 1.16; 95% CI, 1.12-1.20) (MacKay et al., 2010). Academic achievement, by 3rd grade reading and maths tests, was investigated in >128,000 8-year-old American children who had been born at full term. Gestational age was significantly and positively related to reading and maths scores, and children born at 37-38 weeks achieved significantly lower scores than those born between 39-41 weeks (Noble et al., 2012).

1.3.5 Intra-uterine growth restriction and low birth weight

1.3.5.1 Introduction

Since antenatal depression has been associated with the adverse obstetric outcomes of IUGR and LBW (described in section 1.2.4.6.2 above) this topic will be described in more detail. A LBW infant may have been born preterm but be normal weight for their gestational age (as detailed in PTB in section 1.3.3 above); alternatively they may have IUGR, whether full-term or preterm,

indicating poor growth. Prior to the 1960s LBW and/or gestational age formed the definition of prematurity; it was not until after this time that the two measures were distinguished by WHO (Fleischman et al., 2010). Consequently, historically there has been lack of clarity in definitions, and terms have been used interchangeably for research on prematurity. This has hindered the understanding of causes, consequences and treatments for adverse obstetric outcomes (Behrman, 2007, Bamberg and Kalache, 2004). The following sections principally concern IUGR.

1.3.5.2 Definitions

IUGR refers to the poor growth of the foetus *in utero* i.e. a pattern of growth deviating from the expected norms. IUGR may be asymmetric (the most common), generally with head sparing, or symmetric. An infant small for gestational age (SGA) has a birth weight below the 10th percentile for gestational age; they may be constitutionally small (non-pathological) or have had IUGR. Low birth weight is defined as <2500g, and is further subdivided into very low birth weight (VLBW) (<1500g) and extremely low birth weight (ELBW) (<1000g).

1.3.5.3 Epidemiology

The prevalence of IUGR is in the region of 8%. IUGR is present in about 50% of stillbirths, additionally IUGR accounts for 10% of perinatal mortality (Mandrizzato et al., 2008).

1.3.5.4 Aetiology

The aetiology may be divided into placental (abnormalities of placental structure or function e.g. chorioangioma, infarction), the most common cause of IUGR; foetal (e.g. chromosomal disorders) or maternal (see below) (Resnik, 2002, Mandrizzato et al., 2008). As for PTB, depression and IUGR have aetiological factors in common, thus aetiology of IUGR is detailed in this section.

1.3.5.4.1 Maternal

1.3.5.4.1.1 Depression

There is a modest association between antenatal depression, IUGR and LBW, the best overall evidence available, and its limitations, has been described for PTB in section 1.2.4.6.2 above. Again more methodologically rigorous research is required to clarify the issue.

1.3.5.4.1.2 Other

IUGR is also associated with (i) behavioural conditions, for example smoking, alcohol and illicit substance use or abuse, (ii) maternal medical conditions, for example vascular disease and hypertension, renal insufficiency, SLE, diabetes and infection, (iii) extremes of reproductive age i.e. <16 years and >35 years, (iv) poor maternal weight gain, (v) malnutrition, (vi) low pre-pregnancy weight, (vii) lower social-economic status, (viii) drugs such as anticonvulsants. Some of these associations are particularly strong, for example IUGR occurs in 50% of pregnant women with heroin addiction and smokers have a 3.5 fold risk of IUGR (ACOG, 2001). Furthermore, many of these maternal factors are independently associated with MDD and antenatal depression (see sections 1.2.2.2 and 1.2.4.3 above).

1.3.5.4.2 Pathophysiology

The mechanisms underlying poor foetal growth are not fully understood but likely include inflammatory response, hormones, growth factors, vasculopathy, vasoactive agents, maternal and foetal blood and foetal central nervous system (Sankaran and Kyle, 2009).

1.3.5.5 Outcome

The outcome depends on the specific cause for IUGR but the risk of morbidity and mortality increases with decreasing birth weight (Pallotto and Kilbride, 2006).

1.3.5.5.1 Foetus

The IUGR foetus is at increased risk of hypoxia (Marsal, 2002) and there is increased risk for sudden unexplained intrauterine death (OR = 7.0; 95% CI, 3.3-15.1); this risk increases with increasing severity of growth restriction (Pallotto and Kilbride, 2006).

1.3.5.5.2 Offspring

In the short-term there is around a 2- to 5-fold increase in risk for perinatal mortality both for full term and preterm IUGR births (Pallotto and Kilbride, 2006). There is also an increased risk of morbidity, including peri- and neonatal hypoxia, hypothermia, hypoglycaemia and other metabolic abnormalities, compromised immune system, infection and cerebral palsy (Marsal, 2002). In the long-term, IUGR offspring with chromosomal disorders, congenital malformation or infection may well have developmental retardation, neurosensory deficits and poor academic achievement.

Offspring with other causes of IUGR have been followed longitudinally; there is increased risk of shorter height and lower weight in childhood and shortness of stature as adults. There is increased risk of cerebral palsy and subtle neurological findings, developmental delay, a modest association with decreased IQ and with childhood behavioural and emotional problems (Pallotto and Kilbride, 2006). In recent years, a growing literature links IUGR with adult disorders such as cardiovascular disease, metabolic disorders and obesity (Barker et al., 1993) (see section 1.5 below).

1.3.5.5.3 Family and society

Having an IUGR child may create considerable emotional and financial stress for families and economic burden for society, much as described for PTB described in section 1.3.3.4.2 above.

1.4 The hypothalamic-pituitary-adrenal axis

1.4.1 Introduction

The HPA axis is a key theme in this thesis; I have assessed the maternal HPA axis in pregnancy and the infant HPA axis in the first year of life, and thus it is described here in more detail. I first review the HPA axis in general (including adult and infant HPA axis) in section 1.4.2; next I focus on the HPA axis in major depression in section 1.4.3, then the HPA axis in pregnancy (including maternal and foetal HPA axis) in section 1.4.4 and lastly the HPA axis in antenatal depression in section 1.4.5. In particular, I have reviewed aspects of the HPA axis such as diurnal variation in cortisol and the cortisol awakening response, since these are measures I have made in this study.

1.4.2 The hypothalamic-pituitary adrenal (HPA) axis

The HPA axis is the principal endocrine system regulating the body's response to stress (see Figure 2). The HPA axis is headed by the peptides corticotrophin-releasing hormone (CRH), and arginine vasopressin (AVP), which are produced in the paraventricular nucleus of the hypothalamus. CRH enters the pituitary portal blood and, at the anterior pituitary, triggers secretion of the polypeptide, adrenocorticotrophic hormone (ACTH). In turn, ACTH enters the peripheral circulation and at the adrenal cortex, stimulates release of the glucocorticoid, cortisol (Dinan and Scott, 2005). HPA axis activity is then regulated by cortisol, which exerts a direct negative feedback, via glucocorticoid (GR) and mineralocorticoid (MR) receptors, at the hypothalamus and pituitary. Furthermore, the HPA axis is regulated by other factors: It responds to psychological, physical and immunological stressors via excitatory and inhibitory inputs at the paraventricular nucleus of the hypothalamus that arise in other brain regions, for example, hippocampus, amygdala, bed nucleus of the stria terminalis, dorsomedial nucleus of the hypothalamus and brainstem nuclei. Lastly, HPA axis activity has an endogenous circadian rhythm, which is regulated by the suprachiasmatic nucleus of the hypothalamus and results in peak HPA axis activity in the morning (Lightman et al., 2002).

Cortisol has many actions that prepare the body to deal with a stressor, for example, gluconeogenesis, peripheral insulin resistance, anti-inflammatory action and elevated blood pressure. Its central actions include regulation of neurogenesis, neuronal survival, neuronal excitability, memory acquisition and emotional evaluation of events (Pariante and Lightman, 2008).

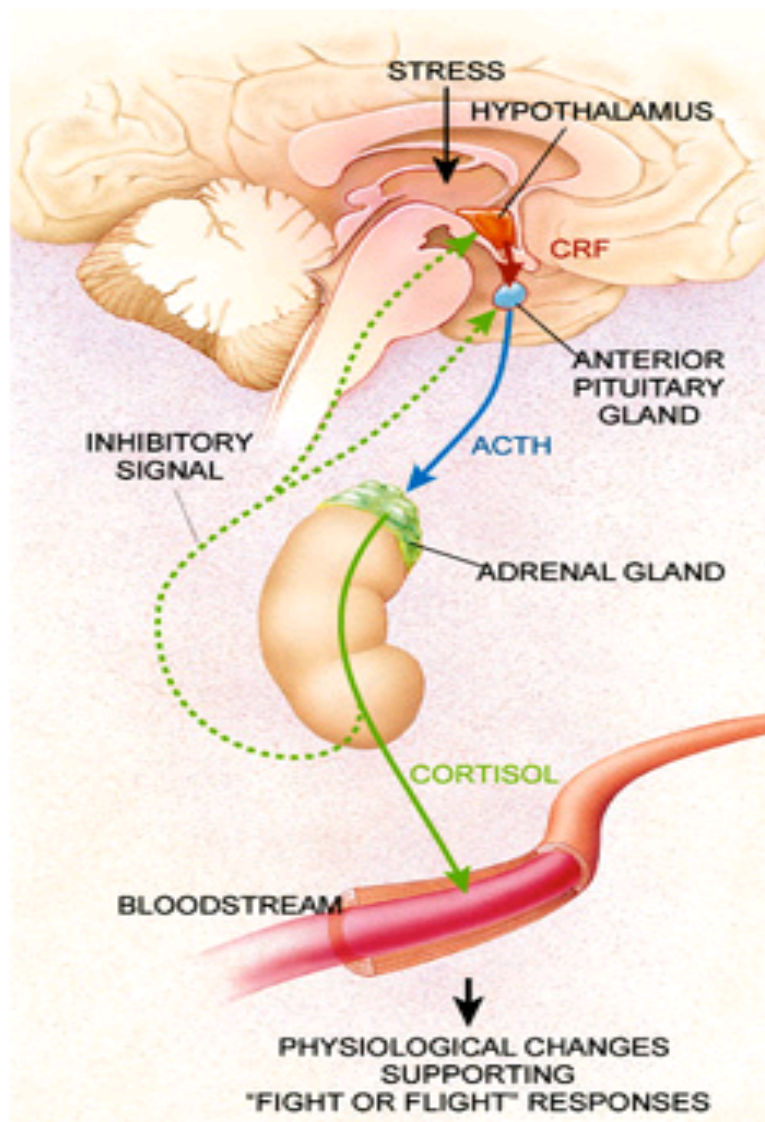


Figure 2: Diagram of the HPA axis

1.4.2.1 Diurnal variation in cortisol secretion

The cells of the suprachiasmatic nucleus (SCN) of the hypothalamus form a biological clock known as the 'master circadian pacemaker,' which creates and maintains circadian rhythms. Creation of these rhythms is endogenous i.e. they are not dependent on, but can be moderated by, external factors such as light. It is hypothesized that circadian rhythms have evolved as an adaptation to the rhythmic environment of the Earth, allowing our physiology and behaviour to align with its daily cycle (Albrecht, 2011, Vaze and Sharma, 2013). One such circadian rhythm is in the diurnal secretion of cortisol; levels are lowest around midnight and rise gradually from 2 to 3am to peak within 30 minutes of waking, levels subsequently decline throughout the day to midnight (see diagram in Figure 3). Only more recently it emerges that this diurnal pattern closely tracks an underlying ultradian rhythm whereby cortisol is released in pulses at approximately hourly intervals. The amplitude of these pulses of cortisol dictates the pattern of its diurnal variation (Lightman and Conway-Campbell, 2010). Clearly the circadian rhythm of cortisol has implications for the timing of sample collection for cortisol measurement.

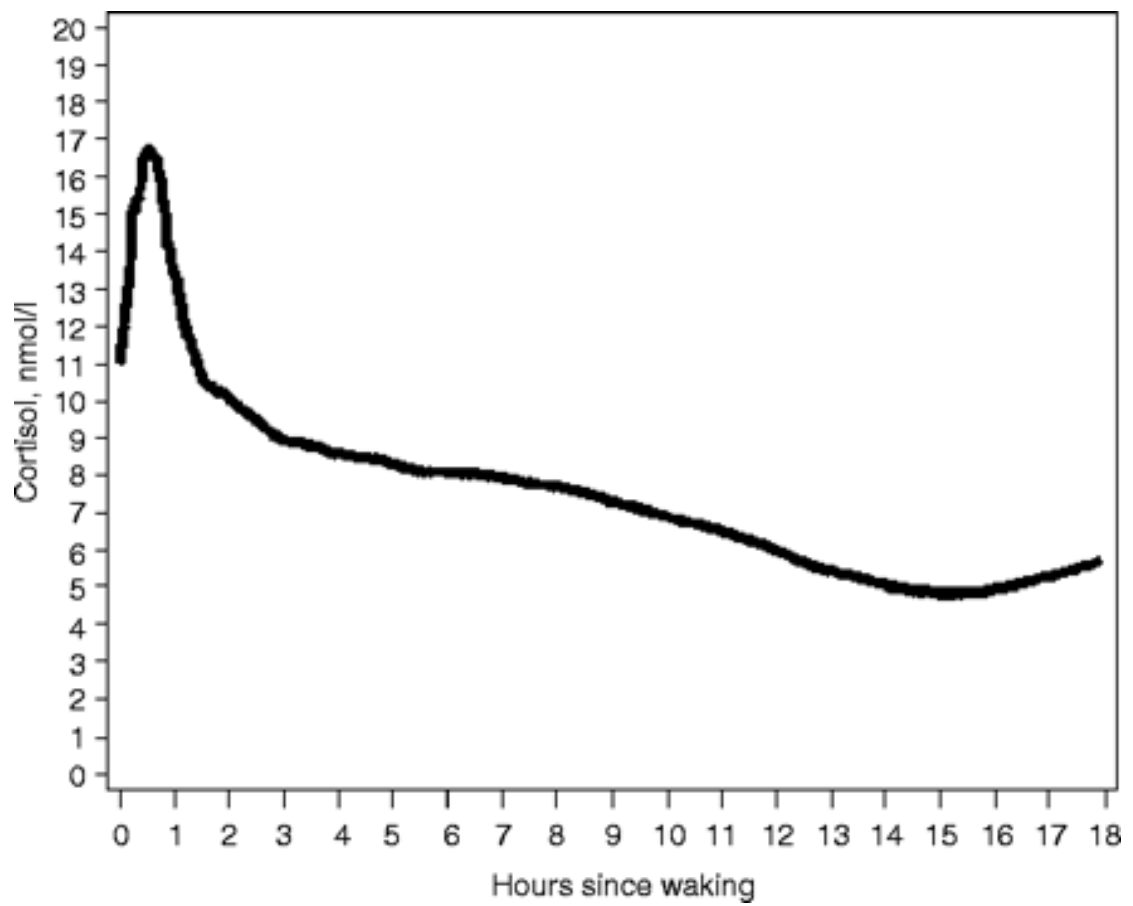


Figure 3: Diurnal variation in cortisol secretion (Ranjit et al., 2005)

1.4.2.2 The cortisol awakening response and post-awakening cortisol

1.4.2.2.1 Introduction

First described in the 1990s, one distinct and separate characteristic of the circadian rhythm of cortisol secretion is the cortisol awakening response (CAR) (Pruessner et al., 1997); a surge of cortisol secretion (a 50-160% increase (Clow et al., 2004)) that occurs on awakening and peaks within about 30 to 45 minutes (see Figure 4). The CAR is thought to be a physiological response to awakening (Clow et al., 2010) and the hippocampus is central to its regulation (Fries et al., 2009). Furthermore, in addition to the negative feedback of glucocorticoids on the HPA axis, another regulator of the CAR is the suprachiasmatic nucleus, both directly via the HPA axis at the hypothalamus and indirectly via extra-pituitary neural pathways to the adrenals that appear to alter the sensitivity of the adrenal to ACTH prior to and after awakening (Clow et al., 2010).

1.4.2.2.2 Function and characteristics of the CAR

The function of the CAR is yet to be understood, but is likely to be manifold. It has been hypothesized to have a role in recovery from sleep inertia, the 15 to 60-minute period between awakening and regaining full alertness, providing a boost of energy (Clow et al., 2010), in cognitive function, regulation of the immune system and anticipation of the up-coming day (Fries et al., 2009). Although previous research suggests that there is high intra-individual stability of cortisol response (Fries et al., 2009) it is now being recognized as an inter-individual state marker, for example, prior day experiences, ovulation, and alcohol consumption (Law et al., 2013). The CAR is seen in 75% of healthy adults and it is about 40% heritable (Wust et al., 2000).

1.4.2.2.3 Factors influencing the CAR

Studies of factors influencing the CAR have provided inconsistent results for depression (Clow et al., 2010) (see section 1.4.3.2 below). Stress appears to be associated with the CAR, although the effect may differ for acute versus chronic stress (Fries et al., 2009); a recent meta-analysis showed that the most robust psychosocial associations with the CAR were job and general life stresses (increased CAR), and fatigue, burnout and exhaustion and possibly post traumatic stress disorder (PTSD) (reduced CAR) (Chida and Steptoe, 2009). Physical diseases, for example cardiovascular and autoimmune disorders, have been associated with a high waking cortisol and blunted CAR (Clow et al., 2010). It seems that age, gender, gonadal hormones and smoking have little, if any, effect on the CAR (Fries et al., 2009). Sleep and waking characteristics may have an

effect, for example, early awakening has been associated with a larger CAR, although duration of sleep and nocturnal awakening has not (Fries et al., 2009).

1.4.2.2.4 Measurement of the CAR and post-awakening cortisol

A number of approaches are used to measure the CAR; serial saliva samples are acquired after awakening and free cortisol is subsequently quantified. Using these methods, the CAR is variously described as repeated measures analysis of cortisol levels from the serial samples, change scores between awakening and peak value, or by calculating the area under the curve (AUC) (Clow et al., 2004). Historically, CAR has been used to describe various aspects of post-awakening cortisol, however, recent expert consensus guidelines propose a clear division. These guidelines suggest that the term CAR should be restricted to measures of the dynamic of post-awakening cortisol changes (for example AUC_i or mean increase in cortisol from awakening). Total post-awakening cortisol (for example AUC_g) may be reported as additional information but is not an estimate of the dynamic of the awakening 'response' (Stalder et al., 2016). Accordingly, this thesis adopts the newly recommended terminology. When the sampling procedure is undertaken in subjects' homes, sample timings may be unreliable (Clow et al., 2004). Although not much implemented in past research, optimal CAR measurement protocols, based on comparative research, have now been proposed. Awakening time can be recorded in the home accurately by electronic means (e.g. the wrist-watch-style actigraph) and sample timings by using an electronic medication event monitoring (MEM) system for saliva collection tubes.(Smyth et al., 2013, Stalder et al., 2016).

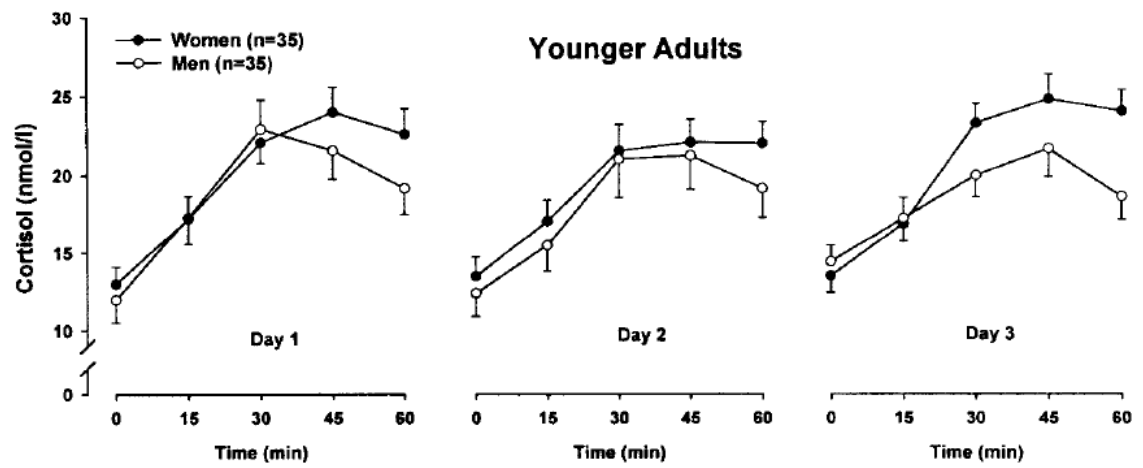


Figure 4: Cortisol awakening response (Pruessner et al., 1997)

1.4.2.3 Neonate and infant HPA axis and infant cortisol response to stress

Since infant HPA axis, including cortisol response to the pain of immunization, is measured in this research, it is now described in more detail. The HPA axis is already functional and stress-responsive in new-borns, although diurnal variation in cortisol is not seen until about 3 months postnatal (Moustogiannis and Vagenakis, 1998). The adult diurnal pattern of cortisol secretion only develops after cessation of daytime naps; prior to this, cortisol levels remain stable from mid-morning to mid-afternoon (Watanura et al., 2004). Measures of HPA axis response to stress in children has received increasing research interest, particularly with emerging evidence that future health and development may be affected by stress during phases of rapid neurological development such as childhood (Gunnar and Quevedo, 2007). Infants are thought to mount a cortisol response to stresses such as pain until around 6 to 12 months-of-age, following this it disappears although a behavioural response persists. Children then develop a period of relative cortisol hypo-responsiveness to stress that diminishes by adolescence (Gunnar and Quevedo, 2007). In their systematic review of cortisol reactivity in children to the age of 2 years, Jansen et al. reported on 48 studies of cortisol response to a range of physical and emotional stressors in children between birth and 2 years of age. There were 35 studies of cortisol response to pain (heel-stick or vaccination); this type of stressor was found to have a large effect size (mean Cohen's d 1.10); only 5 of these studies were in infants older than 6 months and these results were mixed, ranging from no effect to a moderate effect size (Jansen et al., 2010).

1.4.2.4 Measures of the HPA axis

The level of hormones of the HPA axis can be measured. Cortisol is measured in tissues such as blood, urine and saliva; either dynamic measures, by stimulation or suppression tests (e.g. dexamethasone suppression test), or by static levels. Corticosteroid binding globulin (CBG) is present in blood, accordingly total cortisol is measured in this tissue; conversely, only the biologically active, unbound or 'free' cortisol is measured in saliva and urine. ACTH can be measured in blood. Other than during pregnancy, when levels are high enough to measure in blood, CRH is measured in cerebral spinal fluid (CSF). Other indices of the HPA axis may be evaluated, for example, the volume of pituitary or adrenal glands on MRI scans.

1.4.3 The hypothalamic-pituitary-adrenal axis in depression

Hyperactivity and impaired feedback regulation of the HPA axis are consistent biological findings in major depression. An analysis of studies testing HPA axis function (by dexamethasone suppression test) in thousands of depressed patients suggests evidence of impaired negative feedback regulation in 44%; with a higher proportion, around three quarters, in the more severely ill (Arana et al., 1985). HPA axis hyperactivity ensues, as evidenced by increased levels of cortisol in urine and plasma (Sachar et al., 1970), increased levels of CRH in cerebrospinal fluid (Nemeroff et al., 1984) and increased volume of pituitary (Krishnan et al., 1991) and adrenal (Rubin et al., 1995, Nemeroff et al., 1992) glands. The impaired negative feedback regulation of the HPA axis found in MDD has been termed glucocorticoid resistance. Impaired function of the glucocorticoid receptor is hypothesized to contribute to this phenomenon and is thought to be key in understanding the pathophysiology of depression (Anacker et al., 2011). Furthermore, MR, or an imbalance between MR and GR, is also implicated, as reviewed by Berardelli (Berardelli et al., 2013) and Pariante (Pariante, 2009). Whether these abnormalities of the HPA axis are simply a consequence or epiphenomenon, or form part of the pathophysiology of depression remains to be clarified. However, it is hypothesized that in MDD, impaired GR function is central, and the high cortisol levels are consequent (Pariante, 2009).

1.4.3.1 Diurnal variation in cortisol secretion in depression

The normal pattern of diurnal secretion of cortisol (described in section 1.4.2.1 above) appears to be disrupted in MDD although debate on the nature of this disruption continues. Depressed inpatients have been found to have higher morning cortisol and a higher flat pattern of cortisol secretion across the day (Holsboer, 2000, Plotsky et al., 1998); although these findings do not seem to hold for the less unwell (Dienes et al., 2013). However a large community study did show a pattern of cortisol secretion similar to the depressed inpatients described above (Vreeburg et al., 2009). Others variously suggest elevated evening cortisol levels in about 50% (Claustrat et al., 1984, O'Brien et al., 2004), a different pattern of cortisol hypersecretion, or no change in the normal diurnal pattern (reviewed by (Herbert, 2013)). Methodological differences, type and severity of depression, are likely to account for some of these differences.

1.4.3.2 The cortisol awakening response and post-awakening cortisol in depression

Studies of cortisol awakening response in depression have provided inconsistent results (Clow et al., 2010, Dedovic and Ngiam, 2015). Depression has been associated with a higher CAR (indexed by a variety of measures, for example AUCi, change in cortisol between awakening and 30 minutes post-awakening, or repeated measures ANOVA) (Aubry et al., 2010, Bhagwagar et al., 2003, Dienes et al., 2013, Ulrike et al., 2013, Vreeburg et al., 2009) and a greater post-awakening total cortisol (AUCg) (Vreeburg et al., 2009); and a blunted CAR (for example indexed by change in cortisol between awakening and 30 minutes post-awakening or the mean of three values of cortisol at awakening, 15 and 30 minutes post-awakening) (Huber et al., 2006, Stetler and Miller, 2005); these inconsistencies have been attributed to the heterogeneous nature of depression and methodologically diverse studies. It appears that in methodologically more rigorous studies, that is, large studies, controlling for confounding factors or where MDD is operationally defined, MDD is associated with a larger CAR and greater post-awakening total cortisol.

1.4.4 The hypothalamic-pituitary-adrenal axis in human pregnancy

The HPA axis plays a pivotal role in the mechanisms maintaining pregnancy, foetal development and in parturition, although additional hormonal, neuronal and immune pathways, both maternal and foetal, participate in these complex functions. In contrast to very low or undetectable levels in the non-pregnant state, CRH in pregnancy is detectable in plasma from the 8th to 10th week of gestation onwards. CRH is produced by the placenta in exponentially increasing amounts, reaching up to 1000 times that of non-pregnant levels (Goland et al., 1988), to about 800 pg/ml in late pregnancy (Figure 5) and peaking at 2000-3000 pg/ml during labour (Kalantaridou et al., 2010); CRH is undetectable in plasma within 24 hours of delivery (Goland et al., 1986). In contrast to the negative feedback regulation of CRH by cortisol at the hypothalamus, placental CRH output is stimulated by cortisol (Robinson et al., 1988): a positive 'feed-forward' (see Figure 6).

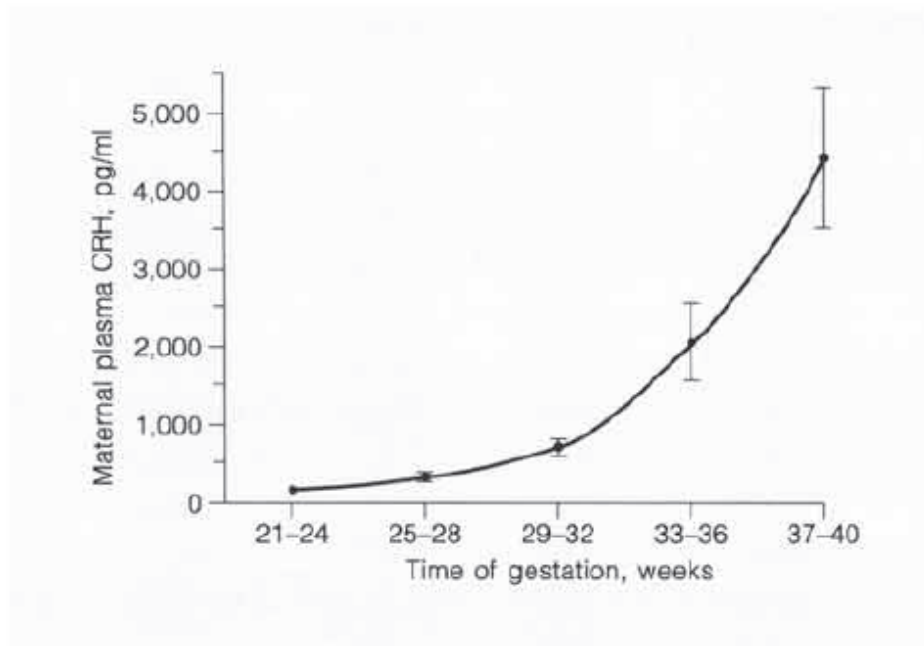


Figure 5: CRH throughout pregnancy, from (Goland et al., 1992)

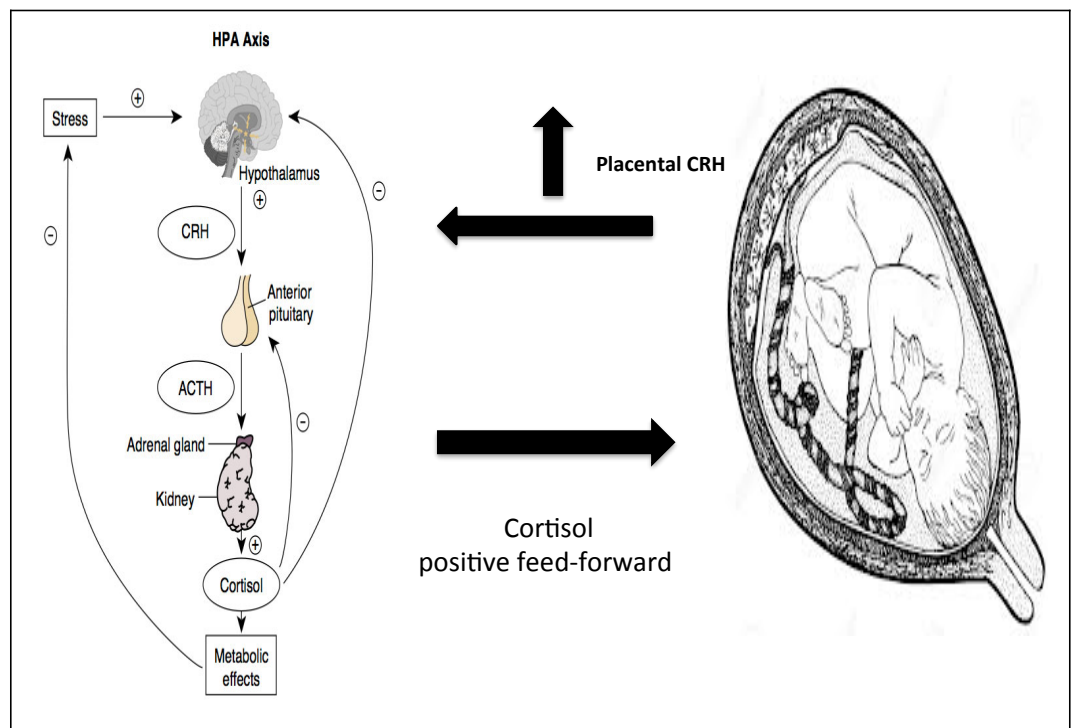


Figure 6: Diagram of gestational HPA axis

CRH exerts its actions by binding to CRH receptors (CRHR1 and CRHR2); it is made inactive by binding to CRH binding protein (CRHBP) which is synthesised in liver and placenta (Petraglia et al., 1993). The level of CRHBP changes little throughout much of gestation until in the 3rd trimester when levels fall, reaching about one third of previous levels within the last 6 weeks, and more-so during labour (Perkins et al., 1993). Within 48 hours of delivery, CRHBP has returned to non-pregnant levels (Campbell et al., 1987).

In pregnancy CRH has multiple targets including placenta, myometrial smooth muscle, foetal membranes and foetal adrenal glands. The role of CRH includes facilitation of embryo implantation (Makrigiannakis et al., 2004), myometrial relaxation and contractility (Grammatopoulos, 2007), control of placental vascular tone (Clifton et al., 1994) and modulation of immune function (Zoumakis et al., 2000) and endocrine function, for example, ACTH (Petraglia et al., 1987), prostaglandins (Jones and Challis, 1989), oestrogen and progesterone (Kalantaridou et al., 2010).

In contrast to unbound CRH, plasma ACTH levels rise throughout pregnancy but stay within normal limits; ACTH levels parallel cortisol levels (Laatikainen et al., 1987). The pituitary gland becomes about 1/3rd larger in pregnancy due to prolactin cell, rather than corticotrophe, hyperplasia. In contrast the adrenal glands enlarge as increasing amounts of cortisol are produced. Cortisol levels peak in the 3rd trimester, total cortisol levels double, and free cortisol levels triple the non-pregnant values; levels are comparable to those found in Cushing's disease or severe depression (Mastorakos and Ilias, 2003).

1.4.4.1 Diurnal variation in cortisol secretion in pregnancy

Diurnal variation of ACTH and cortisol secretion is present in pregnancy (Nolten, 1980, Lindholm and Schultz-Moller, 1973, Kivlighan et al., 2008, Allolio et al., 1990), however CRH does not show a circadian rhythm (Magiakou et al., 1996a, Allolio et al., 1990). Some studies have shown correlations between CRH, cortisol and ACTH in pregnancy, whereas others have not (Mastorakos and Ilias, 2003, Wadhwa et al., 1997). Since ACTH and cortisol both show continued circadian rhythmicity in pregnancy, it is proposed that the ACTH secretagogue, arginine vasopressin, drives these circadian rhythms (Magiakou et al., 1996a).

1.4.4.2 The cortisol awakening response and post-awakening cortisol in pregnancy

The cortisol awakening response is present in pregnancy. In a study of 119 pregnant women, the CAR was present and robust at 32 weeks gestation, with greater mean difference between awakening and 30 minutes post-awakening compared to the non-pregnant state in the same women (de Weerth and Buitelaar, 2005). In three reports all from the same group, one study of 148 pregnant women showed the CAR (measured at weeks 17 and 31 gestation) to decline over pregnancy (Entringer et al., 2010). The second reported a study of 101 pregnant women demonstrating the same attenuation of CAR from 17 to 31 weeks gestation together with the finding of associations between shorter length of gestation and both a reduced attenuation of the CAR over pregnancy and a larger CAR in late pregnancy (Buss et al., 2009). The third reported a study of 25 pregnant women, showing that a flatter CAR occurring with a higher awakening cortisol level was associated with shorter length of gestation, stating that a 2.6% higher awakening cortisol predicted a 1 week shorter length of gestation (Entringer et al., 2011). It is not clear if there was overlap in the subjects used for these three reports. In a study of 94 pregnant women, post-awakening cortisol (AUCg) explained 20% of the variance in birth weight (Bolten et al., 2011). Conversely, in a study of 135 pregnant women, compared with women who had experienced non-sexual abuse or no abuse in childhood, those who had experienced sexual abuse in childhood showed an increasing CAR over the last 2 trimesters of pregnancy (Bublitz and Stroud, 2012). Research on CAR in pregnancy is in its infancy, and since current evidence suggests that the CAR is affected both in depression and in pregnancy, and may have associations with adverse obstetric outcomes, it is relevant to this thesis.

1.4.4.3 Human foetal HPA axis

The foetal HPA axis is critical for foetal development, maturation and homeostasis. An understanding of foetal HPA axis is of relevance to this research, in particular when considering potential mechanisms for developmental programming, and is thus now described. It is known that the structural components of the HPA axis develop early in gestation and the hormones of the HPA axis are present in the 1st trimester. Post mortem studies of human foetuses have shown the presence of (i) ACTH in pituitary tissue at 8 weeks, with a 3-fold higher content of ACTH in foetuses of mid- compared with early gestation (Pavlova et al., 1968) (ii) cortisol in adrenal tissue at 8 weeks (Goto et al., 2006) (iii) CRH immunoreactivity in hypothalamus at 12 weeks (Ackland et al., 1986). Further findings from post mortem studies suggest functional activity: (i) adrenal

CRH1 receptor mRNA, and CRH-stimulation of adrenal cortical cell cortisol production, suggesting direct action of CRH at the foetal adrenal gland, in 16-31 week fetuses (Jaffe et al., 1998), (ii) GR and MR expression in the hippocampus of preterm (from 24 weeks gestation) neonates who died within 4 days of delivery (Noorlander et al., 2006), (iii) a higher concentration of cortisol in the umbilical artery than vein in 16-20 week old live fetuses prior to termination of pregnancy, providing evidence that the foetus is producing cortisol (Partsch et al., 1991). Evidence also exists of foetal HPA axis activity from *in-vivo* studies (i) foetal cortisol response to foetal blood sampling or intra-uterine transfusion demonstrates that HPA axis activity has begun by 20 weeks gestation and that foetal cortisol response is independent of maternal cortisol (Gitau et al., 2001), (ii) CRH has been detected in 2nd trimester foetus umbilical vessel blood; paired maternal plasma samples showed CRH at a higher but positively correlated level (Economides et al., 1987, Lockwood et al., 1996), however (iii) foetal stress response evidenced by a rise in foetal cortisol and ACTH did not include a rise in CRH, suggesting that foetal CRH emanates from another source, likely the placenta (Gitau et al., 2004).

The above studies of Gitau demonstrated that foetal cortisol levels are about 13-fold lower than the maternal level, although about one third of variation in foetal cortisol is attributable to maternal cortisol (Gitau et al., 2001). Maternal cortisol is rendered inactive to the foetus by the actions of the placental enzyme 11- β hydroxysteroid dehydrogenase type 2 (11 β -HSD-2), nonetheless about 10-20% of maternal cortisol crosses to the foetal compartment (Benediktsson et al., 1997). It is estimated that the foetus is exposed to 5% of any elevation of maternal cortisol (Gitau et al., 1998). Placentas from term and preterm deliveries were used to demonstrate that the expression of 11 β -HSD-2 increases throughout pregnancy (Schoof et al., 2001) and drops abruptly at term (Murphy and Clifton, 2003); this facilitates maturation of foetal organs by maternal glucocorticoids.

1.4.4.4 The HPA axis and pregnancy outcome

As discussed in detail in section 1.3 above, the HPA axis plays a pivotal role in foetal growth and development, maintenance of pregnancy and parturition (Mastorakos and Ilias, 2003). To summarize data on HPA axis and obstetric outcome, overactivity of the gestational HPA axis, including elevated CRH (McLean et al., 1995) and cortisol (Giurgescu, 2009), larger CAR in later pregnancy (Buss et al., 2009) and decreased attenuation of CAR over pregnancy have been associated with shortened length of gestation and/or PTB. Lower birth weight has been associated with post-awakening cortisol (Bolten et al., 2011), IUGR with antenatal prescription of

steroids (Mongelli and Gardosi, 2000) and PTB and IUGR in Cushing's disease (Kamoun et al., 2014). CRH in early third trimester has been shown to predict length of gestation (Wadhwa et al., 1998).

1.4.5 The hypothalamic-pituitary-adrenal axis in antenatal depression

The hypothalamic-pituitary-adrenal axis in antenatal depression is a principal focus of this thesis and is thus reviewed in detail below. A number of studies have investigated the HPA axis in antenatal depression, although the HPA axis has not been the primary focus in some of these studies. The majority of these studies have not defined cases of major depression in pregnancy; rather they have used self-report depression rating scales. The studies based on depression symptom scores rather than operationally defined MDD are reviewed first in section 1.4.5.1 below and those based on operationally defined MDD are then reviewed in section 1.4.5.2 below.

1.4.5.1 HPA axis and symptoms of depression in pregnancy

To date, eight studies (six from the same research group) using depression symptom scores rather than operationally defined MDD suggest an association between depressive symptoms in pregnancy and HPA axis (see Table 1). One of these studies assessed CRH and seven assessed cortisol. The majority of the studies demonstrating higher levels of HPA axis hormones compared groups with high v low depression symptom scores. In contrast, six studies using depression symptom scores rather than operationally defined MDD suggest no association between depressive symptoms in pregnancy and HPA axis (see Table 2). Two of these studies assessed CRH and five assessed cortisol; all but 2 of these studies were based on correlations rather than group differences (high v low) of depression scores.

To summarise, the majority of currently available research demonstrates that maternal antenatal HPA axis measures do not correlate with depressive symptom severity scores, but differences in HPA axis measures become apparent when cut-off scores suggestive of clinical depression are used. On consideration of the seven studies that defined a depressed group according to self-rated symptom severity scores, 5 studies showed a group difference suggestive of overactivity of the HPA axis; three measured cortisol in a morning urine sample in the second or third trimester of pregnancy (Field et al., 2004b, Field et al., 2001, Lundy et al., 1999). One (Peer et al., 2013) measured CAR and total post-awakening cortisol (no group difference) and evening saliva cortisol

at a mean of 19 weeks gestation, the other (Rich-Edwards et al., 2008) measured serum CRH between 26 and 28 weeks gestation. Two of the studies that defined a depressed group according to self-rated symptom severity scores found no group difference; one of them measured plasma CRH at <20 weeks and 24-29 weeks gestation (Meltzer-Brody et al., 2011) and the other measured CAR between 25 and 33 weeks gestation (Shea et al., 2007).

Regarding the opposing findings for CRH, both studies were based on a large sample of pregnant women ($n > 800$) with similar socio-demographic characteristics (which were controlled for in the analyses), and both studies recruited subjects from antenatal clinics on the East Coast of the USA. However, there were differences that may account for the opposing findings. The first may be related to differences in the proportion with depression symptom scores above cut-off for antenatal depression: 8.8% with EPDS ≥ 13 (Rich-Edwards et al., 2008) v 24.8% with CES-D ≥ 17 (Meltzer-Brody et al., 2011). The former appears to be more in line with prevalence rates for antenatal depression described in the literature (see section 1.2.4.3 above). Furthermore, both studies also rated symptoms of depression postnatally; in the former study, the proportion with EPDS ≥ 13 at 6 months postnatal was 7.5% (similar to the antenatal rate of 8.8%) but in the latter study, rather than CES-D, EPDS was used at 4 months postnatal and identified 7.6% with EPDS ≥ 13 (in contrast to the antenatal depression rate of 24%). Given these points, it is possible that the latter study may have falsely identified antenatal cases, which might account for the absence of an association with CRH. Indeed, a study of screening for perinatal depression used both instruments (EPDS and CES-D) on the same perinatal women found that a statistically significant greater proportion of women scored above cut-off on CES-D than EPDS (Mosack and Shore, 2006). Furthermore, the study of Rich-Edwards et al. took account of pregnancy complications and prescription medications including antidepressants; this was not the case in the study of Meltzer-Brody et al. Lastly, in contrast to that of Rich-Edwards, the latter study did not use the gold standard RIA to quantify CRH, instead using an enzyme-linked immune-assay, which may also have affected the findings (Glynn and Sandman, 2014). Based on these factors, it seems that Rich-Edwards et al. report more compelling findings of increased CRH in antenatal depression.

The second study reporting no association between depression and HPA axis measured CAR by the change in cortisol from awakening to +30 minutes (Shea et al., 2007). This study divided the

subjects who they had defined as depressed (EPDS ≥ 13 and/or MADRS ≥ 9) into those with or without comorbid anxiety based on STAI score, no differences were found in CAR between the three groups, however, the comorbid groups had higher depression scores than the depression-alone group and there was no report of findings for a combination of those two groups. Furthermore, it was reported that depression scores were statistically significantly lower at the time when the cortisol was measured than at recruitment, so it seems probable that some of the cases groups may not have had depression. Moreover, the influence of other potential confounding factors was inadequately accounted for, since there was no report of indices of social-economic status, ethnicity, obstetric complications, medical conditions or medications, other than that 14 of the depressed women were taking antidepressant medication. Lastly, another study found no difference in CAR, indexed by the change in cortisol from awakening to +30 minutes or in post-awakening cortisol (AUCg) (Peer et al., 2013) (although a higher evening cortisol was reported in the depressed group, as described above). This paper shared the last author with that of Shea et al., (2007) although surprisingly, the findings from that paper were not discussed. Although Peer et al. examined the influence of potential confounding factors more adequately, there were only 8 cases of depression, and these cases were defined by a lower cut-off score (≥ 12) on EPDS. Thus there are a number of limitations to the two current studies of CAR and depression (based on depression symptom score severity); indeed, both papers state that their HPA axis findings should be interpreted with caution.

Table 1 Summary of studies showing an association between symptoms of depression and HPA axis in pregnancy

	Number of subjects	Depression measure	HPA axis measure	Gestational age at sampling	Results
(Diego et al., 2006)	98	CES-D	Urine cortisol	Between 16 and 29 weeks	Positive correlation between CES-D and cortisol
(Field et al., 2001)	120	CES-D	First morning urine cortisol	At first ultrasound appointment	Depressed group (by undefined CES-D score) had higher cortisol.
(Field et al., 2004a)	140	CES-D	First morning urine cortisol	20 weeks	Group with CES-D ≥ 17 had higher cortisol.
(Field et al., 2006c)	430	CES-D	Morning urine cortisol	20 and 32 weeks	Positive correlation between CES-D and cortisol at 32/40.
(Field et al., 2006b)	300	CES-D	Urine cortisol	20 weeks	Sample split into high or low cortisol, the high cortisol group had higher CES-D scores
(Lundy et al., 1999)	43	CES-D	Urine cortisol	Between 27 and 35 weeks	Group with CES-D ≥ 17 had higher cortisol.
(Peer et al., 2013)	53	EPDS	CAR and evening saliva cortisol	19 weeks	Group with EPDS ≥ 12 had higher evening cortisol
(Rich-Edwards et al., 2008)	800	EPDS	Plasma CRH	Between 26 and 28 weeks	Group with EPDS ≥ 13 had higher CRH.

Table 2 Summary of studies showing no association between symptoms of depression and HPA axis in pregnancy

Author	Number of subjects	Depression measure	HPA axis measure	Gestational age at sampling	Results
(Davis et al., 2007)	247	CES-D	Early afternoon saliva cortisol	19, 24 and 30 weeks	No correlation between CES-D and cortisol.
(Meltzer-Brody et al., 2011)	1230	CES-D	Serum CRH	At <20 and 24-29 weeks	Negative correlation between CES-D and CRH. No group difference in CRH using CES-D cut-off >17.
(Pluess et al., 2010)	66	EPDS	Saliva cortisol	10-20 and 32-34 weeks	No correlation between EPDS and cortisol.
(Salacz et al., 2012)	79	BDI	Morning plasma cortisol	Between 36 and 38 weeks	No correlation between BDI and cortisol
(Shea et al., 2007)	66	EPDS or MADRS ¹ and STAI	Awakening saliva cortisol & CAR	28 weeks	No group differences (using either EPDS ≥13 or EPDS ≥13 and STAI ≥40) in awakening or delta cortisol.
(Yim et al., 2009)	100	9-item CES-D	Plasma CRH, ACTH and cortisol	At 5 points between 15 and 37 weeks	No correlation between CES-D and HPA axis measures.

¹ Montgomery-Asberg Depression Rating Scale

1.4.5.2 HPA axis and operationally defined MDD in pregnancy

To date, eight studies, three from the same research group, compared HPA axis measures in pregnant women, either healthy or with an operationally defined diagnosis of MDD. Five of these studies found significantly higher cortisol in the depressed group (Evans et al., 2008, Field et al., 2009, Field et al., 2007, O'Connor et al., 2013b, O'Keane et al., 2011), one found mixed results (Parcells, 2010) and two found no association (Field et al., 2010, Hellgren et al., 2013). Each study is described below; the studies of O'Connor (O'Connor et al., 2013b) and Hellgren (Hellgren et al., 2013) are described in sections 1.4.5.3 (diurnal cortisol) and 1.4.5.4 below (CAR).

In their study of antenatal depression and the HPA axis, O'Keane et al. studied 65 pregnant women either with or without a SCID (Structured Clinical Interview for DSM-IV Axis I Disorders) diagnosis of MDD; they measured saliva cortisol at awakening and 8pm at 32 weeks gestation; compared to healthy controls (n=33) they found significantly higher mean evening cortisol in those with MDD (n=13). Additionally they made fuller assessments of the HPA axis and demonstrated, compared with the control group (n=36), significantly higher plasma CRH in the MDD group (n=19) at 25 weeks gestation and a trend for higher CRH at 36 weeks gestation, but no group differences for ACTH (see Table 3) (O'Keane et al., 2011). This study was undertaken in the same setting as this current research and informed the protocol; however it is on an entirely separate group of study subjects.

In their study of pregnant black women and birth outcome, Field et al. reported on 131 with, and 205 without a SCID diagnosis of MDD in pregnancy. Cortisol in first morning urine was measured at 20 and 32 weeks gestation. Compared to the women without MDD, the MDD group had significantly higher cortisol at 32 weeks gestation (M, 10.5 and 71.0 respectively, $F=6.69$, $p=0.01$). Neither standard deviations, nor the units of cortisol measurement were reported in this publication (Field et al., 2009).

Table 3: Antenatal depression and HPA axis measures - derived from O'Keane et al. 2011

	Control, M (SD) (n = 33-36)	MDD, M (SD) (n = 17-20)	Statistical test
2nd trimester CRH (pg/ml), M (SD)			
	287.22 (79.26)	334 (86.75)	t₍₅₃₎ = 2.04, p < 0.05
3rd trimester CRH (pg/ml), M (SD)			
	546.45 (248.3)	627.21 (223.7)	t ₍₄₉₎ = 2.02, p = .07
2nd trimester ACTH (pmol/l), M (SD)			
	4.49 (2.31)	4.83 (1.4)	t ₍₅₄₎ = 0.73, p = 0.47
3rd trimester ACTH (pmol/l), M (SD)			
	6.45 (2.5)	7.61 (2.3)	t ₍₄₉₎ = 1.6, p = 0.11
Awakening saliva cortisol (nmol/l), M (SD)			
	17.17 (3.93)	16.63 (7.9) ¹	t ₍₄₄₎ = 0.28, p = 0.78
Evening saliva cortisol (nmol/l), M (SD)			
	4.81 (2.0)	7.13 (3.9) ¹	t₍₄₄₎ = 2.67, p = <0.02

¹ n = 13

In their study of sleep disturbances in depressed pregnant women and their newborns, Field et al. studied 253 pregnant women, of whom 83 had a SCID diagnosis of MDD or dysthymia (80% and 20% respectively). Cortisol in first morning urine was measured at 20 and 32 weeks gestation. Compared with the control group, the depressed group had significantly higher urine cortisol at 20 weeks gestation (M (SD), 137.1 (86.4) v 154.8 (89.4) respectively; $F=4.8$, $p=0.05$). Again, the units of cortisol measurement were not reported in this publication (Field et al., 2007).

In their study of cortisol in antenatal depression and anxiety, Evans et al. reported on 182 pregnant women (123 healthy controls, 16 with MDD or dysthymia, 34 with current anxiety disorder and 9 with both MDD and anxiety according to a SCID diagnosis). Saliva cortisol was measured between 10.30am and 11.30am at 36 weeks gestation. The comorbid group, but not the other groups, had significantly higher cortisol than the controls. Cortisol values were not reported in the text but on interpretation of the graph, mean cortisol was approximately 4ng/ml for controls and 5.5ng/ml for the comorbid group. Of note, the cases groups were small and combining the depression groups was not reported, however, depression scores were significantly higher than controls in all three groups. The study included appropriate analyses of potential confounding factors (Evans et al., 2008).

Another study with operationally defined MDD reported a mixed picture of association between MDD in pregnancy and overactive HPA axis. In a study of depression, anxiety and stress during pregnancy, Parcels reported on 59 women in the early- and mid-third trimester of pregnancy, according to a SCID diagnosis, 32% of the sample had MDD at 26-28, and 23% at 32-34 weeks gestation. Cortisol was measured from saliva samples obtained between 10am and 11.30am. There was no statistically significant difference in cortisol levels between the depressed and non-depressed group. However, levels of self-reported symptoms of depression were not high and were similar between the 2 groups. When the groups were defined by a cut-off of symptom severity on BDI, those with the higher level of symptoms had significantly higher cortisol (Parcells, 2010).

In contrast, one study with operationally defined MDD found no association between MDD in pregnancy and overactive HPA axis. In their study of the effect of comorbid depression and anxiety on pregnancy and neonates, Field et al. reported on 911 pregnant women (345 non-

depressed or no diagnosis, 77 anxiety disorder, 181 depressive disorder and 308 comorbid anxiety-depressive disorder). Cortisol was measured in a mid-morning urine sample at 20 and 32 weeks gestation. There was no significant difference in cortisol level between any of the groups at either time point in pregnancy. Limitations include no reporting of the use of prescribed medication, alcohol or illicit drugs nor the awakening time. (Field et al., 2010).

1.4.5.3 Diurnal variation in cortisol secretion in antenatal depression

In a study of 101 pregnant women at high psychosocial risk for developing depression, those with a diagnosis of depression had significantly lower morning cortisol and a flatter slope and higher cortisol level across the day at twenty and thirty-two weeks gestation (O'Connor et al., 2013b).

Another study (described in section 1.4.5.1 above) of 53 women at high psychosocial risk for developing depression compared women with high or low levels of depressive symptomatology; they found higher levels of evening cortisol in women with high levels of depressive symptomatology (Peer et al., 2013); this demonstrates a group difference in the diurnal pattern of cortisol secretion since there was no statistically significant group difference in the awakening levels.

1.4.5.4 The cortisol awakening response and post-awakening cortisol in antenatal depression

The study of O'Connor described in section 1.4.5.3 above found no significant association between antenatal depression and CAR at twenty or thirty-two weeks gestation (O'Connor et al., 2013b). These findings reflect those of other studies: In a study (n = 134) using diagnostic interview, CAR and post-awakening cortisol were measured in late pregnancy (36-39 weeks gestation), no association with MDD was apparent (Hellgren et al., 2013). As described in section 1.4.5.1 above, using a self-rated depression score above or below a cut-off level, CAR was measured between 25-33 weeks (n = 66) (Shea et al., 2007), or <27 weeks (n = 53) gestation (Peer et al., 2013); neither found a difference between groups.

1.4.5.5 Summary of hypothalamic-pituitary-adrenal axis in antenatal depression

In summary, there is mixed evidence for an association of HPA overactivity and depression in pregnancy and there are a number of limitations to the current evidence. The majority of studies use self-report measures of depressive symptoms rather than operationally defined diagnoses,

many of these studies have not been designed specifically to elucidate the relationship between HPA axis and depression in pregnancy, and there are a number of methodological issues such as controlling for potential confounding factors, including antidepressant medication, use of illicit drugs and alcohol, pre-pregnancy BMI, timing of sample collection, pregnancy factors and physical health. On balance, it appears that the larger and/or methodologically superior studies, in particular, those making a specific timed quantification of an HPA axis measure and using diagnostic interview to define the cases groups, for example O'Connor et al. (2013) and O'Keane et al. (2011), or adequate assessment of, and control for potential confounding factors and a large number of subjects, for example, Rich-Edwards et al. (2008), lend support to the hypothesis that the HPA axis is overactive in depression, in particular, evening and diurnal cortisol and CRH. However, the evidence regarding awakening cortisol, CAR and post-awakening cortisol in antenatal depression is limited and further study is warranted.

1.5 Developmental programming

1.5.1 Introduction

Since developmental programming is a principal focus of this thesis, the concept and current evidence is now described. First I introduce the concept, next I focus on studies in animals including research paradigms, outcomes and mechanisms in section 1.5.2. Lastly I have reviewed developmental programming studies in humans in section 1.5.3, beginning with the effects of prenatal stress in general, next the specific effects of antenatal depression and finally a review of proposed mechanisms.

The hypothesis that adversity in the environment of the developing foetus may influence the occurrence of chronic disease in adulthood has developed over the past three decades since the influential epidemiological studies of Barker et al. which demonstrated a link between geographical infant mortality rates or birth weight, and death in adulthood from ischaemic heart disease (IHD) (Barker and Osmond, 1986, Barker et al., 1989). Initially, the investigators concluded that processes linked to growth in prenatal or early postnatal life strongly influence the risk of ischaemic heart disease. These findings are corroborated by other studies in Europe and North America (Buck and Simpson, 1982, Notkola and Tiedeseura, 1985, Forsdahl, 1977). The concept developed that foetal and early-infancy undernutrition induces permanent changes in offspring organ structure, physiology and metabolism leading to ischaemic heart disease in adulthood. In the 1990s it became recognised further that the *in utero* environment afforded not only by maternal nutrition, but also by maternal hormones or metabolism can permanently programme offspring structure and physiology (Barker, 1995). Within the past decade the depth of knowledge has further advanced, providing clear indication that exposure to undernutrition, stress or excess glucocorticoids during foetal development leads to permanent effects on offspring cardiovascular system (CVS), metabolism and cognition (Meaney et al., 2007, Harris and Seckl, 2011). Indeed it is now postulated that glucocorticoids are the final common factor linking the effects of maternal undernutrition, stress and 11 β -HSD-2 activity with programming outcomes of low birth weight with its associated chronic medical problems in adulthood (Cottrell et al., 2012). It is also apparent that the effect of developmental programming is dependent on the tissue, the type of environmental adversity and the timing of its occurrence during foetal development (Harris and Seckl, 2011).

Developmental programming has been demonstrated in humans and other animals and its occurrence is thought to confer evolutionary advantage. Foetal life is a period of rapid growth and development; during this phase there is plasticity i.e. the development of foetal organs and systems is adapted by their environment; later, there is loss of plasticity and systems are fixed. This 'developmental plasticity,' appears adaptive in evolutionary terms as the function of the developed organism is well matched to the environment in which it developed (West-Eberhard, 1989, Glover and Hill, 2012). Although this is likely to promote survival to reproductive age, this 'programming' may be less advantageous to the organism later in life, particularly if the environment at that stage is dissimilar to that which shaped early development (Harris and Seckl, 2011). The evidence regarding mechanisms and outcomes of developmental programming pertaining to glucocorticoids and prenatal stress attained from animal, as opposed to human, studies is most advanced and since it has greatly informed the research in humans, both are described in sections 1.5.2 and 1.5.3 below.

1.5.2 Studies in animals

The study of developmental programming in animals is described first, since it has informed and advanced the understanding of this concept in humans. One advantage of studying animals is the ability to design experiments with strictly controlled environments and the use of experimental manipulations that would be impossible in human studies; this provides clearer clues as to mechanisms and outcomes. The majority of these studies have been in rodents, mainly rats, but also mice and guinea pigs; other species include sheep and non-human primates. However, despite many similarities with humans, for example, about 90% of rat genes (Gibbs et al., 2004) and 99% of mice genes (Gunter and Dhand, 2002) have a human equivalent, differences should be borne in mind. For example, much neural and endocrine maturation occurs after birth in rats and mice, as they are born at an earlier developmental stage (Matthews, 2002); for instance, brain development at birth for rats is equivalent to that of the end of the second trimester in humans (Bayer et al., 1993); furthermore only primates have placental CRH (Weinstock, 2008).

1.5.2.1 Paradigms for animal research in developmental programming

Since the concept of developmental programming is based on the premise of the effects on offspring of an adverse environment during the early development of an organism, three major

paradigms have been employed in the study of this phenomenon in animals: maternal undernourishment, antenatal administration of synthetic glucocorticoids and prenatal stress.

Prenatal stress has been administered throughout, or at specific times in pregnancy, and achieved by methods such as tail suspension, repeated electric shocks, crowding, noise or restraint in rodents and noise in monkeys. Furthermore, additional experimental manipulations have informed the understanding of mechanisms of developmental programming; natural HPA axis activity has been abolished by maternal adrenalectomy and replacement of resting levels of corticosterone (CORT) in pregnant rats. Thus, these rats are unable to mount an HPA axis response to stress and their offspring have then been compared with control prenatally stressed rats (Barbazanges et al., 1996). Finally, in this paradigm, the HPA axis response to stress has been mimicked by injection of CORT at the time of stress administration so that three groups of offspring can be compared. These paradigms provide an understanding of mechanisms for offspring neurodevelopment, health and behaviour as described in sections 1.5.2.2 to 1.5.2.2.4 below.

1.5.2.2 Prenatal stress in animals

1.5.2.2.1 Offspring HPA axis

Prenatally stressed, compared to control rats, show differences in HPA axis reactivity, including higher glucocorticoid secretion at the end of a light period in both sexes, and greater total daily glucocorticoid secretion in females (Koehl et al., 1999). Prenatally stressed male rats and mice have abnormal feedback regulation of the HPA axis with down-regulation of MR and GR (Koenig et al., 2005, Chung et al., 2005, Maccari et al., 2003). Overall, the evidence indicates that prenatal stress in rodents can disrupt offspring HPA axis when it is of sufficient intensity and applied at critical stages of development (Weinstock, 2008). Furthermore, there are similar findings in other species (Schneider et al., 2002, Clarke et al., 1994, de Vries et al., 2007); for example, compared to controls, prenatally stressed juvenile offspring of rhesus monkeys showed higher basal levels of ACTH and cortisol and a larger cortisol response to stress. Moreover, these alterations in offspring HPA axis regulation appear to be permanent (Welberg et al., 2000) and can even persist to the next generation (Schopper et al., 2012). Cross-fostering experiments have shown that these changes originate from prenatal rather than postnatal causes (Schneider et al., 2002, Del Cerro et al., 2010).

1.5.2.2.2 Offspring behaviour

Prenatally stressed animals show altered behaviour. This includes depressive-like behaviour, anxiety and impaired coping in adversity (see below, reviewed by (Weinstock, 2008). Animal models of depression include immobility in the forced swim test (which informs about learned helplessness or loss of active coping) and decreased sucrose preference (for anhedonia) (Czeh et al., 2016). These paradigms have been used to demonstrate an increase in depressive-like behaviours in prenatally stressed (particularly female) rodents (Alonso et al., 1991, Alonso et al., 2000, Keshet and Weinstock, 1995). Furthermore, some studies have shown that depressive-like behaviour in prenatally stressed rats is associated with altered HPA axis reactivity (Morley-Fletcher et al., 2003, Weinstock, 2008, Maccari et al., 2003, Poltyrev et al., 1996), although others have shown that it is not (Solberg et al., 2003, Stohr et al., 2000). Animal models of anxiety can be demonstrated using the elevated plus maze or open field which illicit fear-like reactions. Both male and female prenatally stressed rats have been shown to display higher levels of anxious behaviour using these paradigms (Dickerson et al., 2005, Estanislau and Morato, 2005, Fride and Weinstock, 1988, Murmu et al., 2006, Poltyrev et al., 1996, Salomon et al., 2011, Vallee et al., 1997, Van den Hove et al., 2005, Ward et al., 2000, Zagron and Weinstock, 2006); anxious behaviour was also seen in prenatally stressed rhesus monkeys (Schneider, 1992, Clarke et al., 1994). As for depressive behaviour, anxious behaviour has been correlated with increased HPA axis activity (Vallee et al., 1997, Ward et al., 2000), but not if the prenatal stress was mild or brief (Fride and Weinstock, 1988, Van den Hove et al., 2005, Richardson et al., 2006). Like the changes in offspring HPA axis, these behaviours can persist to the next generation (Matthews and Phillips, 2010).

1.5.2.2.3 Offspring cognitive function and brain development

Several studies have demonstrated learning and memory deficits in prenatally stressed rats and mice (Gue et al., 2004, Ishiwata et al., 2005, Lemaire et al., 2000, Nishio et al., 2001, Son et al., 2006, Wu et al., 2007, Yang et al., 2006). Animal studies (in rodents, sheep and primates) have provided clear evidence that prenatal stress affects offspring brain morphology, and are comprehensively reviewed by Charil et al. The brain regions studied thus far have been chosen by dint of their association with the behavioural or cognitive effects seen in prenatally stressed animals and include both micro- and macroscopic changes, for example, decreased tissue

volumes or neurogenesis; the brain areas studied include hippocampus (Barros et al., 2006, Lemaire et al., 2000, Uno et al., 1994, Uno et al., 1990) amygdala (Kerchner et al., 1995, Kraszpulski et al., 2006, Salm et al., 2004), corpus callosum (Coe et al., 2002), neocortex (Barros et al., 2006, Fleming et al., 1986, Poland et al., 1999), cerebellum (Ulupinar and Yucel, 2005, Ulupinar et al., 2006) and hypothalamus (Anderson et al., 1985, Kerchner and Ward, 1992, Rhees et al., 1999). Although the mechanisms for these effects remain unclear, the placenta and maternal and foetal HPA axes are the principal candidates hypothesized to be the mediators (Charil et al., 2010).

1.5.2.2.4 Offspring physical health

Adverse health outcomes have been demonstrated in prenatally stressed rats including adverse cardiovascular response to stress (Igosheva et al., 2007) and impaired glucose tolerance (Lesage et al., 2004). A wealth of physiological/health effects (for example on blood pressure, glucose tolerance, birth weight and organ development) in offspring exposed to prenatal stress or synthetic glucocorticoids is reviewed by (Meaney et al., 2007).

1.5.2.3 Mechanisms for developmental programming

Since clear evidence exists, in several species (Matthews, 2002), that developmental programming occurs, and prenatal stress has a range of effects on offspring HPA axis, behaviour and health, attention has turned to elucidating the underlying mechanisms for this phenomenon. As described in section 1.5.2.1 above, experimental manipulations employed in studying developmental programming include maternal glucocorticoid exposure, prenatal stress, malnutrition or hypoxia (Xiong and Zhang, 2013). Since it is the focus of this thesis, and it is considered that glucocorticoids are the final common factor linking the effects of maternal under-nutrition, stress and 11 β -HSD-2 activity with programming outcomes (Cottrell et al., 2012), the HPA axis is now considered. Several lines of evidence implicating the HPA axis in the programming effects of prenatal stress are explored in sections 1.5.2.3.2 to 1.5.2.3.5 below.

1.5.2.3.1 Prenatal stress and maternal HPA axis

Firstly, stressing a pregnant animal affects not only the offspring, as described in the preceding section, but also activates the maternal HPA axis. Prenatal stress has been shown to lead to elevated CORT (Takahashi et al., 1998, Williams et al., 1999, Ward and Weisz, 1984, Weinstock

et al., 1988) and decreased corticosterone binding globulin in the pregnant rat (Takahashi et al., 1998) and elevated cortisol in the pregnant rhesus monkey (Schneider et al., 1999). Significantly, maternal glucocorticoids and CRH both reach the foetal brain (Weinstock, 2005), thus activation of maternal HPA axis by prenatal stress will affect the foetal environment.

Secondly, elegantly designed studies in rats further demonstrate the role of maternal glucocorticoids in mediating the long-term effects of prenatal stress on offspring HPA axis activity. For example, pregnant dams were administered restraint as a prenatal stressor; one group with intact CORT secretion and two groups of adrenalectomized dams with replacement steady-dose CORT, one group of which also received an injection of CORT during stress (mimicking the control condition). Whereas prenatally stressed adult offspring displayed a prolonged CORT response to stress and decreased hippocampal MR and GR, this effect was not seen in offspring whose adrenalectomized mothers could not mount a CORT response to the restraint stress, but was reinstated in the group receiving CORT injection during the stress (Barbazanges et al., 1996, Wilcoxon and Redei, 2007, Maccari et al., 2003). Using the same paradigm, others have demonstrated the role of maternal glucocorticoids in mediating the long-term behavioural effects of prenatal stress. For example, in adrenalectomized dams given resting levels of CORT (who were thus unable to mount a CORT response to stress), anxious behaviour in their offspring was prevented (Zagron and Weinstock, 2006) but reinstated when CORT was injected to the dam during the prenatal stress (Oliveira et al., 2006, Salomon et al., 2011, Wilcoxon and Redei, 2007). Furthermore, elevated anxiety displayed in prenatally stressed adult rats is associated with increased plasma CORT stress response (Vallee et al., 1997) and increased CRH in the amygdala (Cratty et al., 1995), and a CRH antagonist diminished anxious behaviour in prenatally stressed rats (Ward et al., 2000).

1.5.2.3.2 Synthetic glucocorticoids

Since synthetic glucocorticoids freely cross the placenta (Seckl and Holmes, 2007) their administration informs on the effects of excess maternal glucocorticoids on offspring. These studies, in sheep, rats, mice, guinea pigs and non-human primates demonstrate lifelong changes in offspring HPA axis regulation (Hauser et al., 2007, Levitt et al., 1996, Sloboda et al., 2002, Sloboda et al., 2007). Furthermore some studies have shown an association between this altered HPA axis regulation and change in glucocorticoid receptor expression in pituitary, hypothalamus and hippocampus in the adult offspring (Banjanin et al., 2004, Liu et al., 2001, Sloboda et al.,

2008). It seems that the long-term consequences on offspring HPA axis, of prenatal exposure to synthetic glucocorticoids depend on the sex of the offspring; females appear more resistant than males (Seckl and Holmes, 2007, Liu et al., 2001). Furthermore, as in prenatally stressed rats, the offspring of rats given synthetic glucocorticoids in pregnancy display an increase in depressive and anxious behaviour (Oliveira et al., 2006). Moreover, effects on health-related physiology, for example glucose intolerance in adulthood (Nyirenda et al., 1998) also persist to the next generation (Drake et al., 2005). To summarise, just like the effects of prenatal stress, administration of synthetic glucocorticoids causes alterations in offspring HPA axis, behaviour and brain development and health-related physiology.

1.5.2.3.3 Placental 11 β -HSD-2

Having established that prenatal stress and maternal or synthetic glucocorticoids have similar programming effects on offspring, attention must turn to the pathway from these factors to the effects in offspring. The first step concerns 11 β -HSD-2, a placental 'barrier' to maternal cortisol. Pharmacological inhibition of 11 β -HSD-2 in pregnant rats results in high exposure of the foetus to CORT; these offspring resemble prenatally stressed rats with overactivity of the HPA axis and depressive and anxious behaviours (Welberg et al., 2000). Furthermore prenatal stress may alter the expression and activity of 11 β -HSD-2 (Mairesse et al., 2007). Much information about the effects of alterations in 11 β -HSD-2 have been gleaned from genetic manipulation of 11 β -HSD-2 in mice, and 11 β -HSD-2 has been proposed as a 'hub' for developmental programming (reviewed by (Cottrell et al., 2014)).

1.5.2.3.4 Glucocorticoid receptors (GR)

The next step is to elucidate the pathway from increased foetal exposure to glucocorticoids to alterations in offspring HPA axis, behaviour and health. Glucocorticoids reaching the foetal brain affect areas with high levels of GR and MR, such as hippocampus and hypothalamus, these are areas critical in the regulation of the HPA axis via CRH. Studies in rodents have shown, in addition to programming effects in offspring, a marked reduction in hippocampal GR expression in association with glucocorticoid exposure or prenatal stress (Levitt et al., 1996, McCormick et al., 1995, Welberg et al., 2001).

1.5.2.3.5 Epigenetics

Lastly, animal research establishes epigenetics as a likely molecular mechanism for programming effects. Epigenetics refers to the reversible regulation of gene expression mediated principally through changes in DNA methylation and/or chromatin structure; epigenetic processes are developmentally regulated and relatively dynamic and thus are a route by which the environment may alter gene expression.

Epigenetic alterations have been demonstrated in the hypothalamus of prenatally stressed when compared with control adult male mice; reduced DNA methylation in the promoter region of the CRH gene and increased methylation in the promoter region of the NR3C1 (GR) gene; there were correlated changes in the expression of these genes in addition to group differences in behavioural and HPA axis response to stress (Mueller and Bale, 2008). Similarly alterations in global methylation were identified following prenatal administration of glucocorticoids in guinea pigs (Crudo et al., 2012). An epigenetic effect of early (a period corresponding to the 3rd trimester of pregnancy in humans) maternal care in rats has also been demonstrated. Compared to adult offspring exposed to low levels of early maternal care behaviours, those exposed to high levels displayed differences in DNA methylation at the exon 1₇ GR promoter in hippocampus in addition to increased expression of hippocampal GR gene (NR3C1) and blunted HPA axis stress responsiveness (Weaver et al., 2004).

1.5.2.4 Animal studies conclusion

In conclusion, many, but not all animal studies have shown that raised levels of maternal glucocorticoids can lead to permanent changes in foetal neuroendocrine development changing the 'set-point' of the offspring HPA axis (Reynolds, 2013), resulting in alterations in development and function of brain areas such as amygdala and hippocampus, in behaviours including depression-like and anxious behaviours and in health-related physiology, as described in sections 1.5.2.2 to 1.5.2.2.4 above. It is clear that there are sex differences in the effects (Weinstock, 2011, Glover and Hill, 2012) and the timing, duration and intensity of prenatal stress or glucocorticoid manipulation is critical in determining the outcome in offspring (Harris and Seckl, 2011).

1.5.3 Studies in humans

As described in section 1.5 above, robust evidence regarding the phenomenon of developmental origins of disease exists in humans. A variety of chronic disorders in adulthood, for example cardiovascular disease, obesity and increased insulin resistance are attributed, in part, to developmental programming (Reynolds, 2013); and evidence accrued over the past two decades points to maternal glucocorticoid excess as a mechanism for this phenomenon. Since the HPA axis and depression in pregnancy are the focus of this thesis, and depression can be conceptualised as a form of chronic stress, the current evidence on these factors as they pertain to developmental programming in humans is now presented.

1.5.3.1 Stress in pregnancy and developmental programming

Studies aimed at understanding processes underlying developmental programming in humans have, thus far, generally focussed on the umbrella term 'prenatal stress'. This term encompasses symptoms of anxiety, pregnancy-specific anxiety, daily hassles, depression symptoms and exposure to life events including severe natural disasters or terrorist attacks.

1.5.3.1.1 Prenatal stress and offspring HPA axis

To date there are relatively few studies published on this topic in humans. Interpretation is limited as a variety of methodologies have been employed, most have quite small numbers of subjects and these studies have not yet been replicated. The diverse types of 'prenatal stress', described above, have been rated by a variety of instruments and at differing gestational ages (Nast et al., 2013). Further variety exists both in the method of offspring HPA axis assessment, for example, basal or diurnal saliva cortisol samples, cortisol and ACTH response to a stressor; and in the timing of HPA axis assessment, ranging from 1 week postnatal to young adulthood. Despite these limitations which hamper a general interpretation, these types of study, reviewed by Glover (Glover et al., 2010), Hunter (Hunter et al., 2011) and Pearson (Pearson et al., 2015) have generally found an association between prenatal stress and offspring HPA axis, specifically elevated basal cortisol or increased cortisol reactivity but again, there is variety in the HPA axis findings.

1.5.3.1.2 Prenatal stress and offspring behaviour and cognitive function

A number of studies have shown an effect of maternal prenatal self-reported anxiety or other stressors on many facets of offspring behaviour, including infants with increased crying/fussing (Wurmser et al., 2006) and decreased attention regulation and temperamental difficulty (Huizink et al., 2002), toddlers with impaired intellectual and language development (Laplante et al., 2004, Bergman et al., 2007), 4-year-olds with behavioural or emotional problems (O'Connor et al., 2002b), 6-year-olds with reduced attention (Gutteling et al., 2006) and school marks (Niederhofer and Reiter, 2004), children and adolescents with emotional problems, attention deficit or hyperactivity (Van den Bergh and Marcoen, 2004). Clearly, there are a variety of behavioural outcomes; these may depend on the gestational stage at which stress occurred (Glover et al., 2010). Furthermore, in contrast to that in rodents, study of the role of genetics and postnatal environment in the development of behavioural outcomes in humans is less advanced. None-the-less, there are data, including a large and elegantly designed study of IVF offspring, either related, or not related to the mother (Rice et al., 2010). Their findings, in the 4-10-year-old offspring, suggested that prenatal stress determined antisocial behaviour; but postnatal, as opposed to prenatal maternal symptoms accounted for offspring anxiety symptoms. However, others found that offspring anxiety was not dependent on postnatal maternal mood (O'Connor et al., 2002b, O'Connor et al., 2002a, Bergman et al., 2007, Van den Bergh and Marcoen, 2004).

1.5.3.2 Depression in pregnancy and developmental programming

Although the relatively small number of studies of the broad term prenatal stress and developmental programming inform on the potential impact of antenatal depression on developmental programming, those specifically concerning antenatal depression are fewer. However, it is an area of importance as depression is common in pregnancy, is recognised to be associated with adverse obstetric outcomes (which are also associated with developmental programming) and is a condition readily diagnosed and treated, thus providing an opportunity for intervention. Since this is a principal focus of this research, antenatal depression and developmental programming are now considered.

1.5.3.2.1 Antenatal depression and offspring HPA axis

To date, there is one published prospective study of exposure to operationally defined depression *in utero* and offspring HPA axis. Field et al. (2010) looked at the effects of depression and anxiety

on pregnancy and neonatal outcome. They compared four groups; healthy controls (n=345), SCID diagnosis of comorbid depression and anxiety (n=308), depression alone (n=181) and anxiety alone (n=77); cortisol was measured in the urine of their 1 to 2 day-old neonates. Compared with neonates of both the healthy and anxiety alone groups, cortisol was higher in neonates of both the comorbid and the depression alone groups. However, although the study benefits from a large sample size, it was hampered by a number of limitations, for example, no account was taken of PTB, birth weight (significant group differences were reported for both these factors), gestational age at birth, mode of delivery or maternal factors, including, antidepressant use. Furthermore, the focus of the studies was on which type, or combination of types, of disorder had worse outcomes and the concept of developmental programming was not mentioned. Moreover, the fact that details of sampling, for example the time of sample collection was not reported, suggests that the HPA axis was a subsidiary rather than a major focus of this study (Field et al., 2010).

Other published studies of antenatal depression and offspring HPA axis use psychiatric rating scale scores rather than operationally defined MDD. Of the current literature, the largest and most recent study is that of O'Donnell et al. who used the ALSPAC cohort (described in section 1.2.4.6.3.3 above). Basal HPA axis activity was assessed by measuring saliva cortisol in 889 15-year-old adolescents at 4 time-points on up to 3 consecutive days. Maternal self-rated symptoms of anxiety (using the Crown Crisp Experiential Inventory (Crown and Crisp, 1966)) and symptoms of depression (using the EPDS) had been ascertained in the second and third trimesters of pregnancy. The main focus of the study was 3rd trimester anxiety, however, symptoms of depression were also analysed. Findings included a modest reduction in cortisol awakening response and a flatter diurnal slope of cortisol secretion in the offspring of women with above cut-off symptoms of depression or anxiety in pregnancy. The effects of these diagnoses were not independent of each other but were independent of potential confounding factors such as lifestyle, psychosocial and obstetric factors, postnatal anxiety and indicators of adolescent health and behaviour. The authors concluded that their study provides strong evidence of an effect of maternal antenatal mood on offspring HPA axis (O'Donnell et al., 2013).

Complementing the basal HPA axis examined by O'Donnell et al., HPA axis reactivity was assessed in a further study on the ALSPAC cohort. On this occasion the effect of exposure to

self-rated symptoms of depression (EPDS) in the third trimester of pregnancy on cortisol response to a carbon dioxide (CO₂) stress test (a physiological challenge) in 139 of the 15-year-old offspring was examined. Those exposed to high levels of symptoms of depression in the third trimester of pregnancy showed a blunted HPA axis response to stress (Vedhara et al., 2012).

In their sample of 116 normal pregnant women and their full-term infants, Davis et al. found no correlation between antenatal symptoms of depression, anxiety or stress and cortisol response to heel-stick procedure in newborns. However, mean psychological assessment scores were low, indicating a psychologically healthy group of subjects (Davis et al., 2011b).

De Bruijn et al. did not distinguish between depression and anxiety in their study of *in utero* exposure to these symptoms; they generated mean depression (EPDS) and anxiety scores from self-ratings in each trimester, including those with EPDS scores above 12 as depressed. They endeavoured to measure basal activity and stress reactivity (to a frustration task) of the HPA axis in the toddlers or preschool children of 51 exposed cases and 52 unexposed controls. They analysed boys and girls separately and found that, compared to the non-exposed, exposed girls, had higher initial, pre- and post- test cortisol; there were no group differences for the boys. The exposed girls showed significantly higher cortisol than the exposed boys for the first two cortisol samples, but there was no group difference between the non-exposed girls and boys. The frustration test did not elicit a cortisol response in either sex, however, although previously shown to elicit distress in toddlers (Calkins and Johnson, 1998) this test has not been shown to elicit a cortisol response in this age-group. Results were only presented for boys and girls separately, not for the whole group (de Bruijn et al., 2009).

In their study of cortisol response to a stress paradigm in 171 6-month-old infants, Brennan et al. assessed *in utero* exposure to depression by retrospectively administered maternal SCID interview. MDD in pregnancy and/or postpartum (n=104) predicted to higher infant cortisol reactivity to stress (Brennan et al., 2008).

In their small epigenetic study, Oberlander et al. found a correlation between second and third trimester symptoms of depression (EPDS score) in pregnancy and increased methylation at a nerve growth factor inducible A gene (NGFI-A) binding site of the GR gene, NR3C1. Furthermore,

increased methylation was associated with increased saliva cortisol response to stress in the 3-month-old infants, although association between antenatal depressive symptoms and infant stress response was not reported (Oberlander et al., 2008).

In their study of the effect of maternal depression on 69 new-borns up to 2 weeks old, Diego et al. showed higher urine cortisol in those born to women with high levels of self-rated symptoms of depression (CES-D cut-off score) at both later second trimester and the very early postnatal period (Diego et al., 2004).

In a similar study from the same group, Lundy et al. showed higher basal urine cortisol in infants (n=35) within 24 hours of delivery in those born to women with high levels of self-rated symptoms of depression (CES-D cut-off score) in the third trimester (Lundy et al., 1999).

Lastly, using the ALSPAC cohort, O'Connor et al. (2005) reported a study of prenatal symptoms of anxiety and HPA axis activity in 10-year-old offspring (n = 74); however they also reported on symptoms of depression in pregnancy. In this study MANOVA established a significant association between anxiety at 32 weeks gestation and offspring awakening cortisol, however there was no report of a similar analysis with prenatal depression. When antenatal symptoms of depression were included as covariates in a regression analysis, symptoms of depression were shown to predict lower awakening cortisol but this finding was dismissed as artefact. Furthermore, as for the other measures of prenatal mood, depression symptom scores were significantly correlated with the rise in cortisol from awakening to 30 minutes. Thus it is difficult to form a conclusion about the effect of symptoms of depression from this study, since depression was not the principal focus (O'Connor et al., 2005).

In summary, despite the variety of methods by which mood and infant cortisol were assessed and the limitations of several of these studies, there is currently evidence to suggest that, as for the broader concept of prenatal stress, depression in pregnancy may have a programming effect on offspring HPA axis from new-born to adolescence. This thesis addresses the current gap in the literature regarding the possible programming effects of operationally defined depression.

1.5.3.2.2 Antenatal depression and offspring behaviour, cognitive function and health

This topic is reviewed in section 1.2.4.6.3 above; to summarize, antenatal depression, or symptoms thereof, has been associated with a wide variety of outcomes in offspring, from neonates to adolescents. These outcomes include (i) behaviour, ranging from poorer performance on NBAS in neonates to symptoms of anxiety, antisocial behaviour, poor attention and depression in older offspring, (ii) cognitive function, with developmental delay and (iii) health indicators, with higher central adiposity.

1.5.3.3 Mechanisms

Although not yet elucidated, mechanisms for the phenomenon of developmental programming in humans have begun to attract research interest in recent years. The appreciation and synthesis of several lines of research lead towards a fuller understanding of mechanisms and are described in sections 1.5.3.3.2 to 1.5.3.3.6 below.

1.5.3.3.1 Prenatal stress and/or depression and maternal antenatal HPA axis

When trying to unravel the potential mechanisms involved in the programming effects of prenatal stress or depression, elucidating their biological effect on the pregnant woman is key. Some studies have demonstrated an overactive maternal HPA axis in stressed pregnant women (Kivlighan et al., 2008, Obel, 2003, Wadhwa et al., 1996), and on balance, the evidence outlined in section 1.4.5 above, suggests that the maternal HPA axis is also overactive in depression in pregnancy.

1.5.3.3.2 Prenatal exposure to glucocorticoids in humans

Administration of synthetic glucocorticoids will effect the regulation of the maternal and foetal HPA axes. Firstly, some synthetic glucocorticoids freely cross the placenta (Seckl and Holmes, 2007) thus the foetus is directly exposed to glucocorticoid, and secondly, in contrast to suppression of the HPA axis by synthetic glucocorticoids in the non-pregnant state, administration of synthetic glucocorticoids stimulate placental CRH production (see section 1.4.4 above); hence the HPA axis is activated (King et al., 2001).

Evidence for altered offspring HPA axis function in infants exposed to synthetic glucocorticoids *in utero* is largely based on preterm infants and suggests suppression of the cortisol response to pain that persists to at least 8 weeks postnatal (Schaffer et al., 2009, Ashwood et al., 2006, Davis

et al., 2006, Davis et al., 2004). However, numerous other stressors associated with PTB interfere with the interpretation of the effect of prenatal glucocorticoids on infant HPA axis (Grunau et al., 2005). Yet, one study assessed healthy infants born at full-term, 30 of whom were exposed to a single course of betamethasone *in utero* and 60 who were not so-exposed; they found, in the exposed group, a larger cortisol response to the heel-stick procedure (which is performed within 24 hours of birth, furthermore, gestational age at exposure to betamethasone was negatively correlated with size of cortisol stress-response (Davis et al., 2011c). These findings are complemented by another study of 209 children born at full term; aged 6-11 years, those exposed to synthetic glucocorticoid *in utero*, showed greater cortisol response to the Trier Social Stress Test for children (more pronounced in female offspring), suggesting that the effects may persist in the longer term (Alexander et al., 2012).

1.5.3.3.3 Maternal antenatal HPA axis and infant HPA axis

Some researchers have shown an association of maternal cortisol in pregnancy and infant HPA axis measures. For example, in a small longitudinal study maternal cortisol was measured in the second trimester of pregnancy. Compared with children of mothers with lower levels of cortisol, those of mothers with higher levels of cortisol showed higher cortisol concentrations following immunizations aged 3-5 years (n=24) (Gutteling et al., 2004) and in response to starting school (n=29) (Gutteling et al., 2005). In a larger study maternal cortisol was measured in 116 women; higher maternal cortisol in late second and the third trimester was associated with a larger cortisol response to a heel-stick procedure within 24 hours of birth. These findings were independent of mode of delivery, prenatal medical history, socio-economic status or child race, sex or birth order (Davis et al., 2011b). Finally O'Connor et al. measured cortisol in amniotic fluid in the second trimester of 125 uncomplicated pregnancies. Cortisol response to stress was measured in the infants at 17 months of age. Those exposed to higher levels of cortisol *in utero* had higher pre-stress cortisol levels and a blunted cortisol response to stress (a separation-reunion paradigm); again these findings were independent of potential confounding factors. Together these data suggest that children's HPA axis function may be predicted from their antenatal exposure to cortisol (O'Connor et al., 2013a). However, correlations across mother and infant HPA axis function could be due to traditional genetic transmission (DNA variants), for example, genetic influences on infant HPA axis function might be inherited by the offspring from the mother in the usual way, leading to correlated biological traits that can manifest as direct genetic effects or be

mediated through heritable epigenetic marks. Animal studies have used paradigms, for example maternal adrenalectomy, to demonstrate that traditional genetic transmission is not mediating offspring HPA axis programming (see section 1.5.2.3.1). Clearly such paradigms cannot be employed in human research, however the 'prenatal cross-fostering' design used by Rice et al. (2010) (described in section 1.5.3.1.2 above) could provide a means of further exploring the genes versus environment question.

Of note, placental CRH is another theoretical mechanism by which foetal exposure to glucocorticoids might be influenced. Most animal studies of developmental programming use rodents, these animals do not have placental CRH, thus placental CRH is not a consideration. However, in humans, in contrast to the negative feedback by cortisol on hypothalamic CRH, placental CRH secretion is stimulated by cortisol; CRH is secreted directly into the foetal compartment, thus bypassing the placental barrier, by 11 β -HSD-2, to cortisol. *In vitro* studies demonstrate that CRH has a direct functional effect at the foetal adrenal (reviewed in section 1.4.4.3 above), however, to my knowledge, there are no studies of maternal CRH in pregnancy and offspring HPA axis. The programming effects of CRH on offspring behaviour and development are reviewed in section 1.5.3.3.4 below.

1.5.3.3.4 Maternal antenatal HPA axis and infant behaviour and development

Just as in studies of prenatal stress or depression, which demonstrate an effect on offspring behaviour and development (described in section 1.5.3.1.2 above), other studies have demonstrated associations between maternal antenatal HPA axis and offspring behaviours. For example maternal antenatal cortisol and CRH in the second trimester of pregnancy and infant negative reactivity at 2 months of age (Davis et al., 2005, Davis et al., 2007), and antenatal cortisol and cognitive development in 1-year-old offspring (Davis and Sandman, 2010). Infants of mothers with high versus low levels of cortisol in late pregnancy displayed more crying and fussing over the first 5 months of life (de Weerth et al., 2003). Higher cortisol in late pregnancy was associated with delay in mental and motor development at 3 to 8 months of age (Huizink et al., 2003).

1.5.3.3.5 Placental 11 β -HSD-2

As outlined in section 1.4.4 above, the placental enzyme 11 β -HSD-2 inactivates a large proportion of maternal cortisol, thus protecting the foetus from glucocorticoid exposure. Evidence from animal research lends support to the role of 11 β -HSD-2 in developmental programming (1.5.2.3.3 above). 11 β -HSD-2 is inhibited by glycyrrhizin, which is found in liquorice; this has provided some indirect evidence in a region (Finland) where consumption of large amounts of liquorice is common. Compared with offspring of those who did not consume liquorice in pregnancy, 8-year-old offspring of women who consumed large amounts of liquorice were shown to have raised saliva cortisol and impaired cognitive and behavioural development (Raikkonen et al., 2009, Raikkonen et al., 2010). Another study demonstrated a negative correlation between symptoms of anxiety prior to delivery and placental 11 β -HSD-2 mRNA expression (O'Donnell et al., 2012). Together, these studies lend weight to the hypothesis that 11 β -HSD-2 is mechanistic in developmental programming.

1.5.3.3.6 Epigenetics

Animal research establishes epigenetics as a plausible molecular mechanism for developmental programming and is described in section 1.5.2.3.5 above. To recap, epigenetics refers to the reversible regulation of gene expression mediated principally through changes in DNA methylation and/or chromatin structure. Epigenetic processes are essential for normal cellular development and differentiation, and allow the regulation of gene function through non-mutagenic mechanisms. Epigenetic processes are developmentally regulated and relatively dynamic and thus are a route by which the environment may alter gene expression. Informed by the animal literature in this field, research in humans has begun to provide preliminary evidence for the role of epigenetics in the broader field of developmental programming. For example, adults who were exposed to famine prenatally had less DNA methylation of IGF2 (insulin-like growth factor) gene than their unexposed siblings (Heijmans et al., 2008). Furthermore increased cytosine methylation of an NR3C1 exon 1_F (GR) promoter (and decreased GR mRNA) was found in the post-mortem hippocampus of suicide victims with a history of abuse in childhood compared with suicide victims with no abuse history and with controls (McGowan et al., 2009). Radke et al. demonstrated persisting epigenetic changes with increased DNA methylation in the GR promoter in 9-11 year old offspring of women who experienced intimate partner violence in pregnancy (Radtko et al., 2011). Lastly, some studies have shown an association between stress or depression in

pregnancy, and epigenetic differences in genes involved in the regulation of the HPA axis (see the examples below). One study of prenatal stress demonstrated a positive correlation between stress and NR3C1 promoter methylation in cord blood DNA (Mulligan et al., 2012). Specifically considering antenatal depression, some small studies have shown an association between severity of symptoms of depression in pregnancy and epigenetic differences in genes involved in the regulation of the HPA axis. An association between methylation of the GR gene (NR3C1) in cord blood DNA and severity of symptoms of depression in the second or third trimester of pregnancy is reported (Oberlander et al., 2008) and a similar finding in buccal cells of 2-month old male, but not female, infants (Braithwaite et al., 2015). Furthermore, in the study of Oberlander et al., the increased methylation of NRC31 was also associated with increased cortisol response to stress in infants at 3 months of age, and the methylation status of this gene was thus hypothesised as a mediating factor linking prenatal maternal mood and infant HPA axis activity (Oberlander et al., 2008). Conversely, Schroeder et al. (2012) explored the association between maternal psychiatric illness and treatment during pregnancy and neonatal DNA methylation patterns in a prospective study of 201 dyads; they found no large effects on neonatal DNA methylation. While this cannot exclude a role for environmental factors on neonatal methylation, it is consistent with there being no strong or global environmental effects on foetal methylation profiles caused by psychiatric illness or psychotropic drugs, although these could be uncovered by larger or more comprehensive epigenetic studies (Schroeder et al., 2012). Interestingly, Teh et al. (2014) demonstrated the importance of gene x environment interactions on the epigenome in their study of genotype and DNA methylomes in the cord blood of 237 neonates whose *in utero* environment had been phenotyped (including symptoms of depression and by other factors known to influence a range of developmental outcomes). They concluded that genotype explained approximately 25% of differences in methylation of the epigenome, but the interaction of genotype and the *in utero* environment explained the remaining 75% of differences. Accordingly, they suggest that in order to avoid underestimating the impact of the environment, studies of the influence of the *in utero* environment on subsequent disease risk should also assess the extent to which environmental influences are moderated by genotype (Teh et al., 2014).

1.5.3.3.7 Human studies conclusion

A solid evidence base demonstrates, in humans, that exposure to an adverse prenatal environment is associated with chronic disease in adulthood. Although it is a relatively new research field in humans, the pursuit of an understanding of the mechanisms for this developmental programming is underway. This quest is much informed by the more advanced findings from animal experiments, which demonstrate that foetal exposure to glucocorticoids mediates the effect of prenatal stress on offspring HPA axis, and molecular mechanisms for this mediation may be epigenetic in nature. In humans, prenatal stress, which includes studies of depression, is a paradigm by which the mechanisms for developmental programming may be further studied and understood. Although these mechanisms are likely to be complex and multifaceted, it seems likely that exposure of the foetus to high levels of glucocorticoids is key and effects on subsequent HPA axis regulation, behaviour, cognitive function and physical health of the offspring result. Epigenetic mechanisms are postulated to underlie the process of developmental programming, however, this line of research in antenatal depression is in its infancy. Lastly, to date, studies of antenatal depression have not incorporated the measures of maternal antenatal HPA axis that would facilitate examination of potential pathways to altered offspring HPA axis.

1.6 Principal research questions

1.6.1 Antenatal depression and the maternal HPA axis in pregnancy

As detailed in section 1.4.5 above, evidence regarding the association of antenatal depression and maternal HPA axis is limited. Only a minority of these type of studies are of operationally defined MDD according to a diagnostic instrument; furthermore, few measure more than one index of the HPA axis across different stages of gestation with adequate control for potential confounding factors, thus further study is warranted. On balance, as in MDD outside of pregnancy, the evidence is suggestive of overactivity of the HPA axis, particularly evening and diurnal cortisol and CRH. The evidence regarding awakening cortisol, CAR and post-awakening cortisol in antenatal depression is limited, however, it is reasonable to hypothesise that findings in pregnant depressed women will mirror the differences found in these measures in non-pregnant depressed v controls, since that appears to be the case for evening and diurnal cortisol, which have been better-studied. Thus, my hypotheses regarding awakening cortisol and CAR are formulated with the additional consideration of HPA axis findings in depression outside of pregnancy (i.e. increased morning cortisol in a meta analysis (Knorr et al., 2010), increased post-awakening cortisol (AUCg) (Vreeburg et al., 2009), and increased CAR indexed by change in cortisol between waking and +30 minutes (Dienes et al., 2013), and a larger increase in cortisol between awakening and +30 minutes measured in pregnant women compared with the same women in the non-pregnant state (de Weerth and Buitelaar, 2005).

1.6.1.1 Hypotheses

1.6.1.1.1 Compared with healthy pregnant women, those with operationally defined MDD will have higher CRH at 25 weeks gestation.

1.6.1.1.2 Compared with healthy pregnant women, those with operationally defined MDD will have lower CRHBP at 25 weeks gestation.

1.6.1.1.3 Compared with healthy pregnant women, those with operationally defined MDD will have higher total (serum) cortisol at 25 weeks gestation.

1.6.1.1.4 Compared with healthy pregnant women, those with operationally defined MDD will have higher free (saliva) cortisol at 25 and 32 weeks gestation, indexed by:

Raised awakening cortisol

Raised evening cortisol

Raised total daily cortisol secretion

Elevated CAR (AUCi) and post-awakening cortisol (AUCg)

1.6.2 Antenatal depression and pregnancy outcome

As described in section 1.2.4.6.2 above, evidence regarding the effect of antenatal depression on obstetric outcome is limited. There have been 2 meta-analyses of antenatal depression and PTB and LBW, which included 1 each of IUGR/SGA, birth weight as a continuum and length of gestation. The majority of published studies are not of operationally defined MDD according to a diagnostic instrument; moreover, much of the data are from studies with methodological weaknesses. This study will contribute to the small amount of evidence derived from studies of operationally defined MDD and obstetric outcome; furthermore potential confounding factors such as maternal smoking, use of illicit drugs and alcohol, medication, medical conditions, age, pre-pregnancy BMI, pregnancy factors and physical health, are controlled for providing a methodologically robust design.

1.6.2.1 Hypotheses

1.6.2.1.1 Compared with healthy pregnant women, those with operationally defined MDD will have a higher rate of PTB, LBW and SGA babies and a shorter length of gestation.

1.6.3 Maternal HPA axis in pregnancy and pregnancy outcome

As described in section 1.4.4.4 above, previous studies have demonstrated that maternal HPA axis measures in pregnancy are correlated with obstetric outcome. For example shortened length of gestation and/or PTB have variously been associated with elevated CRH, elevated cortisol, a larger CAR in later pregnancy and decreased attenuation of CAR over pregnancy.

1.6.3.1 Hypothesis

1.6.3.1.1 HPA axis measures (CRH, CRHBP, awakening, evening and diurnal cortisol secretion, CAR and post-awakening cortisol) will be correlated with obstetric outcomes (PTB, length of gestation, LBW and SGA).

1.6.4 Antenatal depression, maternal HPA axis and pregnancy outcome

As described in sections 1.2.4.6.2 and 1.4.5 above, although antenatal depression is associated with adverse obstetric outcomes, and furthermore, the presence of an overactive HPA axis in antenatal depression is suggested, there are no studies of operationally defined antenatal depression and the role of the HPA axis in obstetric outcome.

1.6.4.1 Hypothesis

1.6.4.1.1 The maternal antenatal HPA axis is a mediator or moderator in the pathway from antenatal depression to pregnancy outcome.

1.6.5 Antenatal depression and offspring HPA axis

As described in section 1.5.3.2.1 above, to date there is only one published prospective study of exposure to operationally defined depression *in utero* and offspring HPA axis, it seems this study was not designed to investigate developmental programming of offspring HPA axis and has a number of important limitations. Studies of depressive symptoms in pregnancy and offspring HPA axis have found a reduction in cortisol awakening response, a flatter diurnal slope of cortisol secretion and a blunted HPA axis response to stress in 15 year-old offspring; higher pre and post stress test cortisol in female pre-school or toddlers and higher urine cortisol in new-borns. In summary, despite the limitations, there is currently evidence to suggest that, as for the broader concept of prenatal stress, symptoms of depression in pregnancy may have a programming effect on offspring HPA axis from new-born to adolescence. This thesis addresses the current gap in the literature regarding the possible programming effects of operationally defined depression.

1.6.5.1 Hypotheses

1.6.5.1.1 Antenatal depression and offspring HPA axis at 8 weeks postnatal

1.6.5.1.1.1 Compared with those not exposed, 8-week-old infants exposed to MDD *in utero* will have a larger saliva cortisol response to pain stress.

1.6.5.1.1.2 Compared with those not exposed, 8-week-old infants exposed to MDD *in utero* will have higher basal saliva cortisol levels

1.6.5.1.2 Antenatal depression and offspring HPA axis at 1 year postnatal

1.6.5.1.2.1 Compared with those not exposed, 1-year-old infants exposed to MDD *in utero* will have a larger saliva cortisol response to pain stress.

1.6.5.1.2.2 Compared with those not exposed, 1-year-old infants exposed to MDD *in utero* will have higher basal saliva cortisol levels

1.6.6 Antenatal depression, maternal HPA axis in pregnancy and offspring HPA axis

As described in sections 1.5.3.3.3 and 1.5.3.2.1 above, although some studies have shown an association between maternal cortisol levels in pregnancy and offspring HPA axis function, and furthermore, studies of depressive symptoms in pregnancy suggest an effect on offspring HPA axis, there are no studies of operationally defined antenatal depression and the role of the maternal HPA axis in offspring HPA axis function.

1.6.6.1 Hypotheses

1.6.6.1.1 Antenatal depression, maternal HPA axis in pregnancy and offspring HPA axis at 8 weeks postnatal

1.6.6.1.1.1 Maternal antenatal HPA axis measures will be correlated with offspring HPA axis measures at 8 weeks postnatal.

1.6.6.1.1.2 The maternal antenatal HPA axis is a mediator or moderator in the pathway from antenatal depression to offspring HPA axis at 8 weeks postnatal.

1.6.6.1.2 Antenatal depression, maternal HPA axis in pregnancy and offspring HPA axis at 1 year postnatal

1.6.6.1.2.1 Maternal antenatal HPA axis measures will be correlated with offspring HPA axis measures at 1 year postnatal.

1.6.6.1.2.2 The maternal antenatal HPA axis is a mediator or moderator in the pathway from antenatal depression to offspring HPA axis at 1 year postnatal

Chapter 2 METHODOLOGY

2.1 Design

This thesis describes a prospective longitudinal observational study designed to test the hypotheses described in section 1.6 above. A control group of healthy pregnant women, and their offspring, were compared with a cases group of women with a DSM-IV diagnosis of MDD in pregnancy, and their offspring up to one year postnatal. A schematic of the study schedule is presented in Figure 7.

The number of subjects varies for some of the measures, as the protocol was revised part way through the study in order to improve the protocol, in terms of procedure and measures, and to improve subject recruitment. Thirty-six subjects (control $n = 25$ and case $n = 11$) were assessed within the original ('pilot') protocol and 46 subjects (control $n = 21$ and case $n = 25$) were assessed within the second ('main') protocol, without overlap. A list of differences (in the inclusion and exclusion criteria, the recruitment process and the some aspects of the study procedure) is presented in Table 47, Appendix A.

2.1.1 Contribution of work by the candidate

The original research idea is attributable to Dr Veronica O'Keane (former head of the Section of Perinatal Psychiatry, Institute of Psychiatry, KCL). With Dr O'Keane's guidance I was responsible for the set-up and conduct of the study. This included writing the study protocol, preparing the documents for Research Ethics and Research and Development and administration of matters pertaining to these boards throughout the study.

I wrote and compiled the assessment schedules. Following liaison with the scientists who were to perform the different analyses of biological samples, I wrote the protocols for handling and processing the samples. I coordinated the collection and storage of all the biological samples throughout the study. I performed all the venepuncture for the original protocol, processed these samples and stored the plasma and serum. I set up and managed the Human Tissue Act (HTA) log for all biological samples and located samples and arranged transport for the analyses that were performed outside of our own laboratory. I inspected all of the results of analyses of the biological samples and determined which were suitable to use in the data analyses.

Using the pilot protocol I undertook all recruitment, baseline and 8 weeks postnatal assessments. I was not able to perform many of the 1-year postnatal assessments due to my maternity leave; however, I trained a colleague to do the assessments in my place and reviewed all of the data on

return from my leave. The neonatal examination (NBAS) was performed by my colleague Dr Susan Pawlby and the infant development (Bayley) assessment was performed by my colleague Ms Susan Conroy. Biological samples were analysed by laboratory colleagues as described in section 2.4.2.3 below.

Following the appointment of my first supervisor, Dr Carmine Pariante, as the new head of Section of Perinatal Psychiatry, I contributed to the revision of the protocol in 2010, and was responsible for training and supervising new researchers who also helped in data collection. I continued to undertake the clinical assessments within the second protocol and, where others performed them, I personally reviewed all diagnostic interviews with that researcher. My colleague Susan Conroy and I designed the databases and undertook the data entry. I cleaned all of the databases, coded the variables and performed all of the data analyses.

2.2 Ethical considerations

The study was granted a favourable opinion by King's College Hospital Research Ethics Committee (ref. 07/Q0703/48). Approvals were also obtained from Research and Development Committees at King's College Hospital and South London and Maudsley NHS Trusts. Participants provided written informed consent for themselves and their offspring.

2.3 Participants

Participant recruitment took place at King's College Hospital, London between 2007 and 2012 and subject follow-up was completed in 2013.

2.3.1 Selection criteria

2.3.1.1 Inclusion criteria

2.3.1.1.1 All participants

- Adult women between 18 and 45 years of age
- Singleton pregnancy
- In late second or early third trimester

2.3.1.1.2 Cases

- DSM-IV diagnosis of Major Depressive Disorder in pregnancy (prior to or at baseline)

2.3.1.2 Exclusion criteria

2.3.1.2.1 All participants

- Uterine anomaly
- Known obstetric complications in the index pregnancy
- Severe or relevant chronic medical conditions for example cardiovascular disease, metabolic or endocrine disorder
- Substance misuse
- Unable to communicate in English

2.3.1.2.2 Controls

- Current or past DSM-IV axis I diagnosis

2.3.1.2.3 Cases

- Any current DSM-IV diagnosis (other than MDD & anxiety disorder)
- History of psychosis or bipolar affective disorder
- Taking antidepressant medication at baseline

2.3.1.3 Rationale for selection criteria

An upper age limit of 45 years was set, as the risk of maternal and foetal complications increases with increasing maternal age. This upper limit was required in order to balance recruitment of sufficient numbers with the increased obstetric risk with higher maternal age; incidence of advanced maternal age has increased in recent years but ≥ 45 years is currently considered, in the obstetric literature, as 'very advanced maternal age'.

Uterine anomaly, obstetric complications and a multiple pregnancy were all exclusion criteria as these factors are associated with an increased risk of PTB.

Participants were recruited in the late second or early third trimester of pregnancy based on previous pilot work (O'Keane et al., 2011) which did not find group differences in HPA axis measures earlier in pregnancy, but allowed HPA axis quantification at two distinct time points in later pregnancy.

Since this was a study of MDD in pregnancy, cases were required to have met DSM-IV criteria for MDD in the index pregnancy, and for appropriate comparison, controls were required to be free from lifetime psychiatric disorder. Ideally cases would not have experienced any lifetime DSM-IV axis I diagnoses other than MDD, but this ideal was balanced with the practicalities of finding such participants, however, those with a history of severe mental illness (psychosis or bipolar affective disorder) were excluded.

The use of antidepressant medication at recruitment was an exclusion criterion as it has been associated with adverse obstetric outcome and furthermore, may affect activity of the HPA axis.

Severe or relevant chronic medical conditions were exclusion criteria because of a link with obstetric complications and/or with HPA axis activity.

Finally participants were excluded if they were not able to comply with the study protocol because of an inability to communicate adequately in English.

2.3.2 Method of selection

2.3.2.1 Controls

A convenience sample of controls was identified from women attending for routine antenatal anomaly ultrasound scan at King's College Hospital.

2.3.2.2 Cases

A convenience sample of cases was identified from midwife and GP referrals to the Perinatal Psychiatry Service of South London and Maudsley NHS Foundation Trust, based at King's College Hospital (KCH).

2.3.3 Recruitment process

The recruitment process for the main protocol is described; the process did not differ substantially between pilot and main protocols.

2.3.3.1 Controls

2.3.3.1.1 Recruitment strategy

The recruitment of controls was achieved by the weekly attendance of a researcher at the antenatal ultrasound department at KCH.

2.3.3.1.1.1 Step 1

The ultrasound list for routine anomaly ultrasound scans was used to identify women's names and to check that they met the inclusion criteria of maternal age, gestational age and singleton pregnancy. Furthermore, since KCH is a regional centre for ultrasound scans, it ensured that the woman to be approached was booked for antenatal care at KCH. Women were approached in the ultrasound department whilst waiting for, or following, their scan. A recruitment script was used to ensure uniformity in the recruitment process. The researcher called out the woman's name, and the contact began by the researcher giving a brief introduction of themselves and explanation for the approach. Women who were prepared to engage further were then provided with a more detailed description of the study and were given the opportunity to ask questions. If they were prepared to consider participation further, they were provided with a concise colour pamphlet about the research and a more detailed 'Patient Information Sheet' and they provided the researcher with a contact telephone number. Lastly all women approached were asked to provide some details confidentially (postcode, age, parity and ethnicity) in order to examine how those who ultimately did participate compared to the sample population.

2.3.3.1.1.2 Step 2

The next stage in recruitment of controls took place within a few days of the initial contact and entailed a telephone call, from the same researcher, to the potential participant. Again a script

was used; having ascertained that the woman was still interested in participating in the research, they were asked a series of questions regarding the inclusion and exclusion criteria (medical disorders, medication, any known problems with the pregnancy and mental health screening). At this stage an appointment was made for the baseline assessment if appropriate, alternatively they were told that they could not be included and were given the reason.

2.3.3.1.2 Recruitment effectiveness and representativeness

The effectiveness of recruitment of controls was recorded with the total number of women who were screened and approached, those who refused to participate, those who subsequently could not be contacted, and those who were ultimately included. Of the 186 women screened and approached, 11% were included in the study as controls (see Table 4). To ascertain if those who were ultimately included were representative of women who were eligible to participate, the groups were compared on ethnicity, age, parity and IMD¹.

Compared with women who were included in the study, those who refused or were not contactable following the initial approach did not differ significantly in age ($z = -1.8$, $p = .07$), parity ($z = -0.4$, $p = .67$), ethnicity ($\chi^2_{(1)} = 3.3$, $p = .07$), or IMD quintiles ($\chi^2_{(4)} = 5.2$, $p = .27$).

2.3.3.1.2.1 Recruitment representativeness for controls within the pilot protocol

The total number of women who were screened and approached was not recorded for the pilot protocol; therefore to gauge the representativeness of these subjects, control women recruited within the pilot protocol were compared with those recruited within the main protocol.

Compared with women who were included in the study within the main protocol, those who were included in the study within the pilot protocol did not differ significantly in age ($t_{(44)} = 0.4$, $p = .73$), parity ($z = -0.0$, $p = .97$), ethnicity ($\chi^2_{(1)} = 0.1$, $p = .76$), or IMD quintiles ($\chi^2_{(2)} = 4.2$, $p = .12$).

¹ Index of Multiple Deprivation (IMD) was derived from the woman's postcode using an online tool (which uses the Ordnance Survey Code-Point Open Feb 2013 and the Office of National Statistics Indices of Multiple Deprivation 2010) from the National Perinatal Epidemiology Unit (NPEU) (<http://tools.npeu.ox.ac.uk/imd/>). The scores, reflecting socio-economic factors, are divided into quintiles representing the level of deprivation (1 (≤ 8.49 (least deprived)), 2 (8.5 – 13.79), 3 (13.8 – 21.35), 4 (21.36 – 34.17), 5 (≥ 34.18 (most deprived))).

Table 4: Recruitment effectiveness and representativeness for controls

CONTROLS	Subjects approached (n = 186)	Refused¹ or subsequently not contactable (n = 96, 52%)	Met ≥1 exclusion criteria² (n = 69, 37%)	Included (n = 21, 11%)	Included in pilot protocol (n = 25)
Age (years), M (SD)	30.3 (5.9)	29.6 (5.5)	30.6 (6.7)	32.3 (5.0)	32.4 (3.8)
Parity, M (SD)	0.8 (1.0)	0.8 (0.9)	0.8 (1.0)	0.7 (0.9)	0.6 (0.6)
Ethnicity % (n)³					
White	56.2 (104)	54.7 (52)	52.2 (36)	76.2 (16)	80.0 (20)
BME	43.8 (81)	45.3 (43)	47.8 (33)	23.8 (5)	20 (5)
Index of multiple deprivation					
IMD quintile 1 (least deprived), % (n)	3.2 (6)	2.1 (2)	5.8 (4)	0	0
IMD quintile 2, % (n)	2.7 (5)	4.2 (4)	1.4 (1)	0	0
IMD quintile 3, % (n)	16.7 (31)	14.6 (14)	14.5 (10)	33.3 (7)	20.2 (5)
IMD quintile 4, % (n)	43.5 (81)	40.6 (39)	49.3 (34)	38.1 (8)	68.0 (17)
IMD quintile 5 (most deprived), % (n)	33.9 (63)	38.5 (37)	29.0 (20)	28.6 (6)	12 (3)

¹ Reasons for refusal included too busy, and not interested.

² The exclusion criteria included age, English not adequate, previous mental health problem, twin pregnancy, medication and medical problem

³ Ethnicity data was not available on all the women who were screened (all subjects screened n = 185, refused or not contactable n = 95)

2.3.3.2 Cases

2.3.3.2.1 Recruitment strategy

The recruitment of cases was achieved by the weekly attendance of a researcher at the referrals meeting of The Department of Perinatal Psychiatry of South London and Maudsley NHS Foundation Trust, held at KCH.

2.3.3.2.1.1 Step 1

At this meeting a researcher screened all referrals in collaboration with the clinical team. A record was kept of any potential participant who was thought may be depressed, and also met the inclusion criteria of maternal age, gestational age and a singleton pregnancy. If the referral was accepted to Perinatal Psychiatry and the appointment with a clinician was to occur before the woman would be 25 weeks gestation the researcher liaised with the responsible clinician about seeing the woman after her appointment in order to discuss the study or where this was not possible asking the clinician to obtain permission from the patient for the researcher to contact them by telephone. If the appointment with a clinician was to occur beyond 25 weeks gestation, the woman did not attend her appointment or the referral was not accepted to the service, then the researcher made telephone contact and used the recruitment script as for controls (described in section 2.3.3.1.1.1 above). In order to maximise recruitment of cases, if it was not possible to make telephone contact, a letter was sent to that individual providing information about the reason for contact and a date that the researcher would visit to discuss the research. If the woman was not at home for that appointment an explanatory letter was left giving a contact number and inviting the woman to make contact should she wish. These attempts to recruit cases were abandoned after the woman reached 30 weeks gestation.

2.3.3.2.1.2 Step 2

When a researcher was able to make contact with a potential recruit, having established that they were interested in participating in the research, as for controls, they were asked a series of questions regarding the inclusion and exclusion criteria (medical disorders, medication, any known problems with the pregnancy and mental health screening). At this stage an appointment was made for the baseline assessment if appropriate, alternatively they were told that they could not be included and were given the reason.

2.3.3.2.2 Recruitment effectiveness and representativeness

As with controls, the effectiveness of recruitment of cases was recorded with the total number of women who were screened and approached, those who refused to participate, those who could not be contacted, and those who were ultimately included. Of the 243 women screened and approached, 10% were included in the study as cases (see Table 5).

To ascertain how those who were ultimately included were representative of women who were eligible to participate, the groups were compared on ethnicity, age, parity and IMD.

Compared with women who were included in the study, those who refused or were not contactable following the initial approach did not differ significantly in age ($z = -1.2$, $p = .19$), parity ($z = -0.9$, $p = .39$), ethnicity ($\chi^2_{(1)} = 2.2$, $p = .14$) or IMD quintiles ($\chi^2_{(3)} = 4.5$, $p = .21$).

2.3.3.2.2.1 Recruitment representativeness for cases within the pilot protocol

As with controls, the total number of women who were screened and approached was not recorded for the pilot protocol; therefore to gauge the representativeness of these subjects, cases women recruited within the pilot protocol were compared with those recruited within the main protocol.

Compared with women who were included in the study within the main protocol, those who were included in the study within the pilot protocol did not differ significantly in age ($t_{(34)} = 0.9$, $p = .39$), parity ($z = -1.3$, $p = .20$), ethnicity ($\chi^2_{(1)} = 0.0$, $p = .89$), or IMD quintiles ($\chi^2_{(3)} = 4.2$, $p = .24$).

Table 5: Recruitment effectiveness and representativeness for cases

CASES	Subjects screened	Refused¹ or not contactable	Met ≥1 exclusion criteria²	Included	Included in pilot protocol
	(n = 243)	(n = 107, 44%)	(n = 111, 46%)	(n = 25, 10%)	(n = 11)
Age (years), M (SD)	29.2 (6.2)	28.3 (6.1)	30.0 (6.1)	29.6 (6.3)	32.3 (3.8)
Parity, M (SD)	1.0 (1.2)	1.3 (1.4)	0.7 (1.1)	0.8 (0.9)	1.2 (0.9)
Ethnicity % (n)³					
White	59.9 (121)	64.4 (56)	58.9 (53)	73.9 (17)	45.5 (5)
BME	40.1 (78)	35.6 (31)	41.1 (37)	26.1 (6)	54.5 (6)
Index of multiple deprivation (IMD)					
IMD quintile 1 (least deprived), % (n)	0.4 (1)	0	0.9 (1)	0	0
IMD quintile 2, % (n)	1.6 (4)	0.9 (1)	2.7 (3)	0	9.1 (1)
IMD quintile 3, % (n)	12.8 (31)	8.4 (9)	16.2 (18)	16.0 (4)	9.1 (1)
IMD quintile 4, % (n)	56.0 (136)	61.7 (66)	54.1 (60)	40.0 (10)	18.2 (2)
IMD quintile 5 (most deprived), % (n)	29.2 (71)	29.0 (31)	26.1 (29)	44.0 (11)	63.6 (7)

¹ Reasons for refusal included too busy, too unwell, and not interested

² The exclusion criteria included English not adequate, no longer pregnant, not depressed (n = 24), history of bipolar affective disorder, medical condition and taking antidepressant.

³ Ethnicity data was not available on all the women who were screened (all subjects screened n = 202, refused or not contactable n=87, met ≥1 exclusion criteria n = 90)

2.3.4 Subject follow-up

2.3.4.1 Subject retention

Subject retention details for the follow-up assessments are presented in Table 6. Subject retention reduced over the course of the study, furthermore the number providing usable samples for measurement of cortisol was fewer than those who were assessed. However, I compared the proportion of cases v controls at baseline with the proportion of cases v controls completing each follow assessment and there was no statistically significant difference at any time point. Furthermore, I compared socio-demographic information for cases in the smallest sets of follow-up (infant cortisol response at immunization at 8 weeks and at 1 year postnatal) with cases at baseline, there were no statistically significant differences. Likewise, I did the same for controls and there were no statistically significant differences. This indicates that despite the reduction in subject numbers over the course of the study, that compared with baseline there was no significant difference in the proportion of cases v controls at each follow-up assessment. Furthermore, both the cases group and the controls group at follow-up were representative of their respective groups (in socio-demographic factors) at baseline.

2.3.4.2 Equity of follow-up procedures

Researchers performing the study interviews at baseline and follow-up were not blind to caseness. However, all other study measures were performed blind to caseness. Delivery details were obtained from obstetric medical notes and so were not subject to researcher bias. Both the neonatal examination and the assessment of infant development at 12 months were performed by researchers blind to caseness. The laboratory analyses were also performed by technicians who were blind to caseness.

Clinical obstetric management of all subjects was naturalistic and undertaken by NHS staff independent of the study. Clinical psychiatric management of cases was also naturalistic and undertaken by NHS staff and was not determined by study protocol.

In an attempt to optimise subject retention over the course of the study for the main protocol, payments (£10 to £20) were made to participants following completion of each part of the study.

Table 6: Subject retention

Assessment	Controls	Cases	Statistic (proportion of cases and controls at baseline v follow-up)
	% (n)	% (n)	
Baseline			
Interview completed	100 (46)	100 (36)	
Usable saliva cortisol sample	43 (20)	50 (18)	$\chi^2_{(1)} = 0.1, p = .76$
Blood sample	93 (43)	92 (33)	$\chi^2_{(1)} = 0.0, p = .95$
32 weeks gestation			
Psychiatric assessment	39 (18)	50 (18)	$\chi^2_{(1)} = 0.4, p = .54$
Usable saliva cortisol sample	87 (40)	72 (26)	$\chi^2_{(1)} = 0.3, p = .58$
Delivery details			
	100 (46)	100 (36)	
Neonate assessment			
	100 (46)	92 (33)	$\chi^2_{(1)} = 0.1, p = .78$
8 weeks postnatal			
Interview completed	98 (45)	83 (30)	$\chi^2_{(1)} = 0.2, p = .62$
Usable infant stress response cortisol sample	78 (36)	50 (18)	$\chi^2_{(1)} = 1.5, p = .21$
Usable infant basal cortisol sample	80 (37)	67 (24)	$\chi^2_{(1)} = 0.3, p = .58$
1 year postnatal			
Interview completed	87 (40)	68 (25)	$\chi^2_{(1)} = 0.4, p = .51$
Usable infant stress response cortisol sample	59 (27)	44 (16)	$\chi^2_{(1)} = 0.5, p = .47$
Usable infant basal cortisol sample	74 (34)	53 (19)	$\chi^2_{(1)} = 0.9, p = .35$

2.4 Data collection

2.4.1 Procedure

A schematic of the study schedule is presented in Figure 7. Briefly, assessments took place in pregnancy at 25 and 32 weeks gestation, the neonatal period and at 8 weeks and 1 year postnatal.

2.4.1.1 Baseline

The baseline assessment took place as close as possible to 25 weeks gestation¹. According to participant preference, assessments were undertaken in the participant's home or work place, in the clinical department of Perinatal Psychiatry, KCH or at the Institute of Psychiatry, KCL. Following a verbal reminder about the study and an opportunity for the participant to ask questions, written informed consent was obtained prior to any study assessments.

2.4.1.1.1 Participant characteristics

A semi-structured interview was devised to collect information on socio-demographics, current medication, medical history, obstetric history and current health behaviours (see Appendix B) and pre-morbid IQ was assessed.

2.4.1.1.2 Psychiatric assessment

This consisted of a diagnostic interview and assessment of symptom severity according to self-rated psychiatric rating scale scores.

2.4.1.1.3 HPA axis assessment

2.4.1.1.3.1 Blood

Blood was taken and processed in accordance with a blood sampling and processing protocol (see Appendix C) in order to measure plasma CRH and CRHBP and serum cortisol. The samples were obtained between 12pm and 3pm at the baseline assessment,² transported to the laboratory on ice packs and processed within 2 hours of venepuncture. To process the blood, samples were

¹ Mean 26.3, SD 1.6, min. 23.9 to max. 31.7 weeks

² When venepuncture was not possible at the baseline assessment the sample was taken (between 12 and 3pm) as close as possible to 25 weeks gestation.

centrifuged at 1500G at 4°C for 10 minutes. Cryovials were used to store aliquots of plasma and serum, which were frozen immediately at -80°C, pending analysis.

2.4.1.1.3.2 Saliva

Six saliva samples were obtained over the course of one day at baseline: at awakening, 15, 30 and 60 minutes after awakening, midday and 8pm. Salivettes containing a polymer swab (Sarstedt, UK) were used. Subjects were given a demonstration, verbal and written instructions and a mechanical timer for sample collection (see protocol in Appendix D and sample collection record in Appendix E). Emphasis was placed on the accuracy of timings and procedure. Subjects were instructed not to eat, drink or smoke in the first hour after awakening or the thirty minutes before sample collection at midday and 8pm. Subjects were asked to complete a collection record on which to record sample timings and protocol deviations. Samples were refrigerated by participants then posted to the research centre at the beginning of the next working week or were collected in person by a researcher. On receipt, samples were checked for completeness against the collection record, then frozen at -20°C pending analysis.

2.4.1.2 Thirty two weeks gestation

This assessment was conducted by telephone and post.

2.4.1.2.1 Psychiatric assessment

This consisted of completion of self-rated psychiatric rating scales.

2.4.1.2.2 HPA axis assessment

As at baseline, six saliva samples were obtained to measure cortisol levels.

2.4.1.3 Birth of the baby

Details of the labour, delivery and neonate were obtained from the maternal summary, which is a standard electronic document prepared by midwives following the birth of a child in the NHS. In cases where this information was not available, for example, a home birth attended by a private midwife, the information was obtained from the mother at the neonatal examination at six days postnatal.

2.4.1.4 Six days postnatal

An assessment of neonate behaviour (NBAS) was performed as close as possible to six days postnatal¹.

2.4.1.5 Eight weeks and one year postnatal

This assessment was made on the day of the infant's routine immunizations at 8 weeks and at 1 year postnatal.

2.4.1.5.1 Participant characteristics

2.4.1.5.1.1 Maternal

A semi-structured interview was devised to collect information on any change in maternal socio-demographics since the previous assessment, current medication and current health behaviours (see Appendix F and Appendix G).

2.4.1.5.1.2 Infant

The same semi-structured interview included questions about the infant including health, medication and feeding details.

2.4.1.5.2 Maternal Psychiatric assessment

As at baseline, the psychiatric assessment consisted of a diagnostic interview (covering the period from previous assessment to the current time); assessment of symptom severity according to self-rated psychiatric rating scale scores was used to corroborate diagnostic data.

2.4.1.5.3 HPA axis assessment

2.4.1.5.3.1 Infant

Infant saliva samples were obtained before and exactly 20 minutes after the routine immunization and at awakening and 8pm on the following day (see protocol in Appendix H and sample collection record in Appendix I). In almost all cases a researcher accompanied the mother and baby to the immunization clinic to ensure accurate procedure and timing for sample acquisition. The researcher then took the samples to the laboratory where they were frozen at -20°C. The

¹ Mean 9.3, SD 6.4, min. 4 to max. 42 days

mother obtained the infant's saliva samples on the following day having received a demonstration, verbal and written instructions.

Care was taken to avoid feeding for 15 minutes before a sample was taken; the time of the most recent feed and nap were recorded. A Salivette and Salimetrics childrens swab (SCS) was used to collect saliva by the researcher on the immunization day. For ease of use, a Sorbette arrow was used for infant saliva collection the following day. As for mothers, a sample collection record was used to record timings and relevant information.

2.4.1.5.3.2 Maternal

As at baseline, six saliva samples were taken by the participant the day after the infant immunizations; cortisol levels were quantified.

2.4.1.6 One year postnatal

An assessment of infant development (the Bayley Scales of infant development) was performed as close as possible to 1 year postnatal.





PREGNANCY		DELIVERY	POSTNATAL	
				
Baseline (25 weeks gestation)	32 weeks gestation	Birth of the baby / neonatal period	8 weeks postnatal	1 year postnatal
Maternal clinical assessment	Maternal clinical assessment		Maternal clinical assessment	Maternal clinical assessment
Maternal HPA axis assessment	Maternal HPA axis assessment		Maternal HPA axis assessment	Maternal HPA axis assessment
		Record of birth outcomes		
		Neonatal behavioural assessment		
			Infant HPA axis assessment	Infant HPA axis assessment
				Assessment of infant development

Figure 7: Study schedule

2.4.2 Measures

2.4.2.1 Participant characteristics

2.4.2.1.1 Socio-demographics

Maternal age was recorded at baseline.

The following were recorded and coded:

- Maternal and paternal ethnicities were described by the participant and coded as 'white' or 'black and minority ethnic' (BME).
- The level of maternal education, according to participant report, was coded as GCSE or Lower and A level or higher.
- Maternal and paternal employment status was coded according to whether or not they were working outside the home.
- Maternal and paternal classification of employment was determined and coded according to the Office for National Statistics (<http://www.ons.gov.uk>) and a variable created stipulating either 'professional or managerial' or 'not professional or managerial.'
- Marital status was coded as 'married or cohabiting' or 'single with or without a partner'.

2.4.2.1.2 Premorbid IQ

Premorbid IQ was assessed at baseline using the Wechsler test of adult reading¹ (WTAR) (Wechsler, 2001). In this test the subject is presented with 50 irregularly spelled words and prompted to pronounce each; the examiner can assess their vocabulary by their ability to pronounce the words and thus estimate their premorbid IQ. WTAR scores have been shown to correlate highly with measures of IQ (<http://www.pearsonclinical.co.uk>). Since maternal IQ is known to be a strong predictor of offspring IQ (Eriksen et al., 2013, Tong et al., 2007), WTAR was performed on the participants in order to control for any group differences found in infant development. Measurement of full scale IQ during an episode of depression could potentially affect the score (Kessler et al., 2013, Sackeim et al., 1992) and thus be a source of bias between the case and control groups; thus an index of IQ in health (premorbid) rather than during an episode of illness was required.

¹ WTAR is valid only for English-speakers indexed by the following: if the participant was born in an English-speaking country, remained in an English-speaking country to at least 9 years of age and attended an English-speaking school; or if born outside an English-speaking country, attended at least 4 years of secondary school in an English-speaking school.

2.4.2.1.3 Obstetric history and risk factors

Obstetric history was determined by participant report at baseline. Data included any previous pregnancies, parity, history of PTB, miscarriage, elective termination of pregnancy, intrauterine death and age at menarche. Current obstetric risk factors were assessed according to problems in the index pregnancy including vaginal bleeding, the severity of that bleeding and urinary tract infection. These data were recorded at baseline and categorical variables were dichotomized for coding.

2.4.2.1.4 Physical health, medication, health indicators and health behaviours

The following measures were recorded at interview in pregnancy and at 8 weeks and 1 year postnatal:

Information about physical health was obtained by checklist and a variable was created for the presence or absence of a chronic medical condition.

Details of current medication were obtained and a variable was created to indicate whether or not the participant was taking regular medication. A second variable was created to indicate whether or not the participant was taking antidepressant medication. A third variable was created to indicate whether or not the participant was taking any steroid medication, either regular or 'as required' (PRN).

Use of tea, coffee, alcohol (dichotomised as ≤ 2 units/week or above) and cigarettes (in pregnancy prior to baseline and current use) were also recorded.

At baseline pre-pregnancy BMI ($\text{height (m)} / \text{wt}^2 \text{ (Kg)}$) was calculated from the height and pre-pregnancy weight, which was generally reported in the subjects hand-held maternity notes.

2.4.2.2 Mental health

Maternal mental health was assessed at interview in pregnancy and at 8 weeks and 1 year postnatal; self-report questionnaires were used to corroborate diagnostic data.

2.4.2.2.1 Diagnostic assessment

Current and past DSM-IV axis1 disorders were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First, 1996).

2.4.2.2.2 Assessment of symptom severity

To corroborate DSM-IV diagnosis of MDD, symptom severity was determined at each interview by the Beck Depression Inventory. Symptoms of anxiety were also rated, by the State-Trait Anxiety Inventory since they are common in MDD.

2.4.2.2.2.1 Beck Depression Inventory

The Beck Depression Inventory (BDI) is a self-rated instrument which measures the severity of symptoms of depression (Beck et al., 1961). The BDI has been widely used in the assessment of depressive symptomatology both clinically and in research. Its use has been validated as a screening test for depression in the pregnant population (Holcomb Jr et al., 1996, Su et al., 2007). The BDI version IA was used for this research, this tool has 21 items each rated 0-3; question 19 regarding weight loss was omitted, thus the possible range of scores was between 0 and 60.

2.4.2.2.2.2 State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) is a self-rated instrument which measures intensity or frequency of a variety of feelings related to anxiety (Spielberger, 1983). It is divided into two sections, one measuring 'state' (intensity of symptoms) and the other 'trait' (frequency of symptoms) of anxiety. The STAI form Y (state) was used for this research, each section has 20 questions scored from 1 to 4, thus the range of possible scores for each section is 20 to 80. The STAI has been widely used in perinatal research although there has been little work towards validating its use as a diagnostic screen in pregnancy (Meades and Ayers, 2011).

2.4.2.2.2.3 Pro-rating missing data

If data were missing for a particular scale, pro-rating was used to correct for this, however, where $\geq 20\%$ of items were missing, data for the whole scale were coded as missing for that subject.

2.4.2.3 HPA axis assessment

2.4.2.3.1 CRH

Plasma CRH was quantified at the pathology laboratory at King's College Hospital, London using a magnetic radioimmunoassay (RIA) kit supplied by Phoenix Pharmaceuticals Inc, California, USA. The Standard operating procedure from the lab is detailed in Appendix J.

2.4.2.3.2 CRHBP

Plasma CRHBP was quantified by Jo Drury, Liverpool Women's Foundation NHS Trust, UK. Enzyme-linked immunosorbent assay (ELISA) methodology was used. The Standard operating procedure from the lab is detailed in Appendix K. Samples were analysed in duplicate in three batches each with an internal control; CRHBP values were thus corrected to minimise inter-assay variation. The minimum reliable value was set at 100 pmol/l.

2.4.2.3.3 Serum cortisol

Serum cortisol, a measure of total cortisol, was quantified at the pathology laboratory at King's College Hospital, London using a magnetic bead ELISA supplied by Siemens Healthcare Diagnostics Ltd, Surrey, UK. The Standard operating procedure from the lab is detailed in Appendix L.

2.4.2.3.4 Saliva cortisol

Saliva cortisol, a measure of free cortisol, was quantified by Dr Patricia Zunszain, Stress, Psychiatry and Immunology laboratory, KCL using a high sensitivity salivary cortisol ELISA kit supplied by Salimetrics Europe Ltd, Suffolk, UK. The Standard operating procedure is detailed in Appendix M. Samples were measured in duplicate where an adequate volume of saliva allowed. The minimal reliably detectable concentration was set at 0.33 nmol/l and the assay is reliable up to 82 nmol/l. The inter-assay co-efficient of variability (CV) ranged from 8-11%, and the intra-assay CV ranged from 6-10%; both were within an acceptable range (<https://www.salimetrics.com>).

2.4.2.3.4.1 Maternal cortisol measures in saliva

2.4.2.3.4.1.1 Awakening cortisol

The awakening cortisol level was used in the analyses if the sample was acquired within 1 hour of waking and the awakening time was between 5am and 10am. Awakening cortisol levels were used alone as an index of HPA axis activity.

2.4.2.3.4.1.2 Evening cortisol

The evening cortisol level was used in the analyses of cortisol if the sample was acquired at 8pm \pm 1hour. Evening cortisol levels were used alone as indices of HPA axis activity.

2.4.2.3.4.1.3 Diurnal cortisol

It was planned to evaluate diurnal cortisol secretion by calculating the area under the curve for awakening, midday and 8pm cortisol levels. Unfortunately the number of participants with a usable cortisol result for each of these three samples was small at every time point across the study (controls $n = 12-17$, cases $n = 8-13$). For this reason, diurnal cortisol is represented by the AUC between the above awakening and evening samples only, since it was preferable to maximise the amount of data available. Notably, the association between the diurnal cortisol as calculated by both methods was highly positively and strongly correlated at each assessment time point (range of $r = .55$ to $.90$, all p values $< .001$). The AUC was calculated using the formula for the area of a trapezoid (Pruessner et al., 2003).

2.4.2.3.4.1.4 Cortisol awakening response

CAR and post-awakening cortisol were only calculated if the subject woke between 5am and 10am, the awakening sample was acquired within 5 minutes of awakening, the second sample was acquired at 15 minutes ± 5 after awakening, the third sample was acquired at 30 minutes ± 10 after awakening and the fourth sample was acquired at 60 minutes ± 15 after awakening. Missing data were not substituted with mean cortisol values or imputations. Cortisol awakening response was evaluated by two indices, AUC_G and AUC_I, which were calculated using the formula for the area of a trapezoid (Pruessner et al., 2003).

2.4.2.3.4.2 Infant cortisol measures in saliva

2.4.2.3.4.2.1.1 Response to the pain of routine immunization

The infant cortisol level was used in the analyses if the second sample was obtained exactly 20 minutes after the immunization.

2.4.2.3.4.2.1.2 Basal cortisol

Samples were obtained on awakening and in the evening, timings were determined by maternal and infant convenience and were recorded for the analysis.

2.4.2.4 Obstetric outcome measures

2.4.2.4.1 Expected date of delivery (EDD)

The dating ultrasound scan report was used where possible, if the information was not available at baseline, EDD was obtained from hospital delivery information. Compared with EDD estimated from the date of the last menstrual period, the dating ultrasound provides a more accurate measure of gestational age, thus EDD (ACOG, 2009).

2.4.2.4.2 Onset of labour

A variable was created to represent onset of labour: either spontaneous or not spontaneous (for example, induced or no labour). This information was required since only women with a spontaneous onset of labour (regardless of the ultimate mode of delivery) were considered in the examination of the effect of antenatal depression on length of gestation. The information was obtained from the maternal summary where possible, otherwise, from the mother at the six day postnatal assessment.

2.4.2.4.3 Other details of the labour

In order to control for the effect of delivery on infant outcomes, a record was made of mode of delivery (vaginal, elective or emergency Caesarean section), duration of rupture of membranes, duration of the labour and the use of analgesia in labour. This information was obtained from the maternal summary where possible, otherwise, from the mother at the six day postnatal assessment.

2.4.2.4.4 Gestational age at birth

Date of birth and EDD were used to calculate the gestational age at birth to the nearest day.

2.4.2.4.5 Birth weight

The weight of the baby at delivery was recorded; this information was obtained from the maternal summary where possible, otherwise, from the mother at the six day postnatal assessment. A variable was also created to indicate if the baby was LBW (2500g).

2.4.2.4.6 Weight for gestational age

A small-for-gestational age index was calculated using a tool from the Perinatal Institute (a national not-for-profit organisation set up to enhance the safety and quality of maternity care (www.pi.nhs.uk)). The centile, based on a population birth weight at term of 3400g, is calculated for each baby, using gestational age at birth, birth weight and sex of the baby.

2.4.2.4.7 Head circumference

The head circumference at birth was obtained from the maternal summary.

2.4.2.4.8 Sex of the baby

2.4.2.4.9 Apgar score

The Apgar score at 1 and 5 minutes after delivery was obtained from the maternal summary. The Apgar assessment comprises an evaluation of gross cardiovascular, respiratory and neurological functions of the newborn; **A**ppearance, **P**ulse rate, **G**rimace, **A**ctivity and **R**espiratory effort are assessed and each is assigned a score of 0-2 giving a range of 0 to 10. The score at 1 minute reflects how well the baby tolerated the birth process, and at 5 minutes how well the baby is doing outside the womb, and is used to aid the decision of whether or not the baby requires immediate medical attention.

2.4.2.5 Neonatal Behavioural Assessment Scale (NBAS)

The NBAS is an examination of a wide range of behaviour of newborn babies up to 2 months of age (Brazelton, 1995). The NBAS comprises of 28 behavioural, and 18 reflex items. It evaluates the baby's capabilities across different developmental areas and how the baby integrates these areas. The examiner endeavours to evaluate the infant's best performance and accordingly provides stepped support in order to achieve this aim. These data are commonly reduced to conceptually or empirically derived clusters representing key developmental areas including regulation of the autonomic system (e.g. breathing and temperature), motor system (e.g. tone, activity level and reflexes) state (e.g. from quiet sleep to full cry, and the transitions between levels of state) and social interaction (e.g. assessing how ready babies are to engage in their new world). In this study six clusters of neuro-behaviour are reported (orientation, motor, range of state, regulation of state and autonomic stability and reflexes).

The NBAS has been widely used in research with studies including those of at-risk infants, the effects of obstetric medication or mode of delivery, prenatal substance exposure, prediction studies and intervention studies (Brazelton, 1995).

The NBAS examination was performed, blind to caseness, by Dr Susan Pawlby, Section of Perinatal Psychiatry, IoP, who is trained and experienced in the use of this measure.

2.4.2.6 Bayley Scales of Infant Development

Bayley Scales of Infant and Toddler Development (Bayley-III) (Bayley, 2005) uses a series of developmental play tasks to assess infant development in cognitive, language and motor domains. This instrument is recognised internationally as one of the most comprehensive tools to assess child development in the first 3 years of life. The assessment was administered by Ms Susan Conroy, Section of Perinatal Psychiatry, IoP, who is trained and experienced in the use of this tool and was blind to caseness.

2.4.2.7 Use of the measures

The primary outcome measures were (i) maternal antenatal HPA axis (CRH, total cortisol, awakening, evening and diurnal free cortisol, cortisol awakening response (AUCi) and post-awakening cortisol (AUCg), (ii) obstetric outcomes (preterm birth, length of gestation, low birth weight and small for gestational age) and (iii) infant HPA axis measures (cortisol response to pain and basal (resting) cortisol). This research is exploratory in nature; hence a large number of comparisons and correlations are tested. The other measures including maternal factors in pregnancy (socio-demographics, obstetric history and risk, physical and mental health, medication and health behaviours), at 8 weeks and 1 year postnatal (socio-demographics, physical and mental health, medication, health behaviours and HPA axis) and infant factors (delivery details, feeding, health, medication, neonatal neurobehaviour and cognitive, language and motor development at 1 year) were measured in order to assess their influence on any group differences observed in the primary outcome measures.

2.5 Data analysis

The statistical analyses were performed in SPSS Statistics Version 21 (IBM Ltd, UK). First, the data were visually inspected using boxplots; where potential outliers were identified, the source data were checked and z-scores were calculated. The data were examined for normality of distribution with the Kolmogorov-Smirnov test and for homogeneity of variance with Levene's test. When necessary, in order to reduce bias, data were transformed prior to analyses and if this procedure was of no benefit, non-parametric tests were used.

Continuous data are presented as mean (M) and standard deviation (SD) and categorical data as percentage (%) and frequency throughout. Standard error of the mean (SE) is presented in graphs. For univariate analyses, group comparisons of continuous data were made using the independent samples t-test, and, for non-parametric data, the Mann-Whitney test was used and the z-score reported. The Wilcoxon signed-rank test, a non-parametric test for repeated measures, allowed examination of the change in infant cortisol at immunization. Pearson's chi-square (χ^2) test of the independence of variables was used for the analysis of categorical data and Fisher's exact test was used when one or more contingency cells yielded an expected count of less than five. Pearson's correlation (r_p) was used for the analysis of association between parametric continuous variables and Spearman's correlation (r_s) was applied to non-parametric continuous variables. The point-biserial correlation (r_{pb}) was used to test for association between a continuous variable and a dichotomous variable (based on ranked scores for non-parametric data). Phi (ϕ) statistics are reported for association between two dichotomous variables.

Statistics are reported to one decimal place except where two decimal places provide additional meaningful information. Two decimal places are used for p values > .05 and three decimal places for p values \leq .05. In tables, significant results are highlighted in bold or by an asterisk (*). In general the p-values reported are calculated by the asymptotic method; however, in accordance with Field (Field, 2013), in non-parametric tests where the sample size is small (<50) the 'exact' significance is presented. Multivariate analyses were conducted using general linear modelling procedures. The level for type I errors was set at 5%. In addition to reporting p values, confidence intervals and effect sizes are reported and discussed in this thesis since they are considered to be useful additional information by which findings can be evaluated (APA, 2008, Field, 2013). The

effect size for group differences was calculated using the formula $r = \sqrt{\frac{t^2}{t^2 + df}}$ for the independent samples t-test and $r = \frac{z}{\sqrt{n}}$ for non-parametric tests.

2.5.1 Moderation and mediation

I hypothesised that group differences in obstetric outcomes and in infant HPA axis measures would be moderated or mediated by maternal antenatal HPA measures. In the following section I present details of the statistical analyses used to test the hypotheses. Moderation and mediation are both regression-based models. In this thesis I have tested for these effects using a macro named 'PROCESS for SPSS and SAS' (version 2.13, Prof. A Hayes, The Ohio State University, USA. <http://www.afhayes.com>), this custom dialogue box was installed to the regression menu of SPSS. PROCESS uses an ordinary least squares (OLS) regression-based framework for estimating direct and indirect effects in mediation models and interactions in moderation models (<http://www.processmacro.org>). PROCESS is a powerful tool and greatly facilitates testing for moderation and mediation, as it is quick and simple to use.

2.5.1.1 Moderation analysis

Moderation describes the situation in which one variable affects the relationship between two others. If moderation of a relationship between an independent (X) and dependent variable (Y) exists, the moderating variable (M) determines when, or the circumstances under which, that effect exists or may be increased or suppressed; for a conceptual diagram see Figure 8. Statistically, moderation is modelled by an interaction between the independent and moderator variables; see Figure 9. The first step to test for moderation uses hierarchical multiple linear regression to ensure that the assumptions for linear regression are met. Next the PROCESS tool was used to test for interactions and to explore any interaction with simple slopes analysis and the Johnson-Neyman technique.

2.5.1.1.1 Exploring the interaction

If an interaction was found, the nature of the moderation was elucidated by simple slopes analysis or the Johnson-Neyman method. In simple slopes analysis the regression equation is produced for the independent and dependent variables at the mean and 1 standard deviation above and below the mean of the moderator variable. The slopes can be compared for statistical significance

and the value and direction of b ; accordingly one can see whether the relationship between dependent and independent variables changes at different levels of the moderator. The Johnson-Neyman technique is more detailed as it computes a regression model for the predictor and outcome at many different values of the moderator (within the bounds of the values of the moderator that were observed in the data); thus revealing zones of values of the moderator at which the association between the predictor and outcome are, or are not significant.

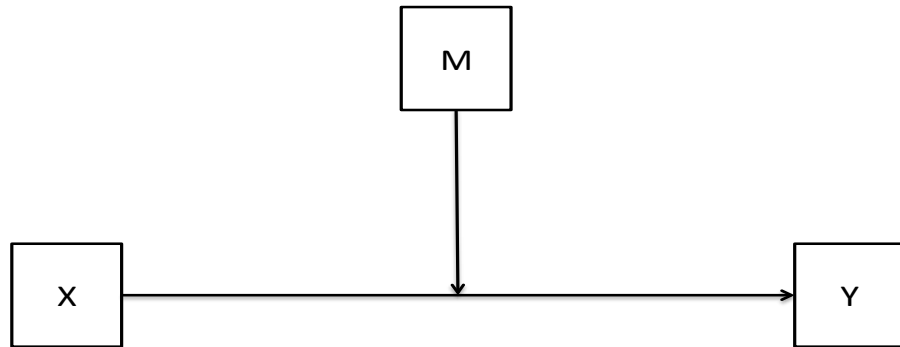


Figure 8: Diagram of a conceptual model for moderation
Note. X independent variable, Y, dependent variable, M moderator variable

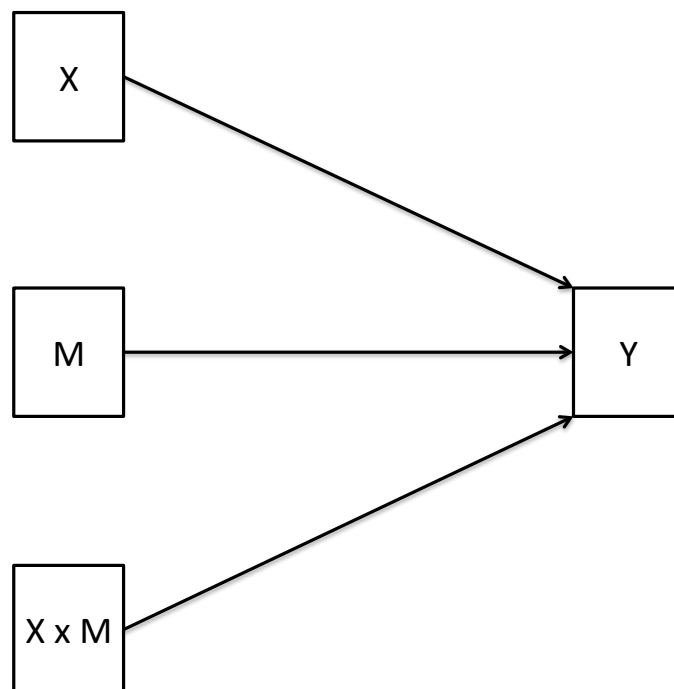


Figure 9: Diagram of a statistical model for moderation
Note. X independent variable, Y, dependent variable, M moderator variable

2.5.1.2 Testing for mediation

In contrast to moderation, which describes *when* an independent variable (X) exerts its effect on a dependent variable (Y), testing for mediation is done by a statistical method designed to elucidate *how* this effect is transmitted. Mediation is said to occur when the independent (X) variable exerts its effect on the dependent variable (Y) via a third intervening or mediating variable (M).

In explanation, the *total effect* of an independent variable (X) on a dependent variable (Y) can be represented by a regression coefficient (path c) see Figure 10. The total effect represents the amount by which two cases that differ in X by one unit are predicted to differ in Y, however the total effect may be reached by *direct* or *indirect* means. In a simple mediation model *a* represents the coefficient for the path from X to M, *b* represents the coefficient for the path from M to Y controlling for X and *c'* represents the coefficient for the path from X to Y see Figure 11. Thus *c'* quantifies the *direct effect* of X on Y, and the product of paths *a* and *b* (*ab*) quantifies the *indirect effect* of X on Y through M. The *total effect* is equivalent to the sum of the direct and indirect effects: algebraically, $c = c' + ab$. The indirect effect (*ab*) represents the amount by which two cases that differ in X by one unit are predicted to differ in Y though X's effect on M, which in turn affects Y. The direct effect (*c'*) represents that part of the effect of X on Y that is independent of M (Hayes, 2009).

Conventional methods of testing mediation models, for example those expounded by Baron and Kenny (Baron and Kenny, 1986) have been criticised¹ and modern approaches, for example those of Preacher and Hayes (Preacher and Hayes, 2004), are now superseding traditional methods (Hayes, 2009). In this modern method, quantification of the size of indirect effect is calculated by regression techniques; an inferential test that does not equal zero establishes that there is indeed an indirect effect. PROCESS uses bootstrap confidence intervals for this inferential test; bootstrapping is a robust method that does not make assumptions about the distribution of the indirect effect; thus it is a non-parametric method (see section 2.5.2 below).

¹ Primarily there is no formal quantification of the indirect effect. Instead the indirect effect is inferred by logic constructed from a series of tests that must all be statistically significant; thus type II error is inflated and power is reduced.

Lastly, Hayes also underscores that, in contrast to conventional methods of testing mediation models, in order to test for an indirect effect, there is no requirement of a simple association between X and Y . Moreover, he reasons that by a failure to test for indirect effects in the absence of a total effect one may miss potentially important mechanisms by which an independent variable exerts its effect on a dependent variable. Furthermore, there is no reliance on statistical significance for any of the individual paths in a mediation model as this does not determine whether the indirect path is significant (Hayes, 2013).

2.5.2 Bootstrap technique

The bootstrap technique is used to estimate the properties of the sampling distribution from the sample data. In explanation, one assumption for parametric tests is that the sampling distribution is normal. Where data are normally distributed it is reasonable to infer that the sampling distribution is normal, however, if the data are not normally distributed this assumption cannot be made; the bootstrap method is a 'robust' measure used to overcome this problem.

In bootstrapping, the sample data are treated as a population from which 'bootstrap' samples are taken; each score is replaced to the sample data before a new bootstrap sample is drawn ('random sampling with replacement'). The parameter (e.g. the mean or b) is calculated in each bootstrap sample. The process is repeated many times (j), for example 1000 to 2000, thus j estimates of the parameter are created. The parameter estimates are rank ordered, accordingly the limits within which 95% of them fall can be discerned, this is known as the 95% percentile bootstrap confidence interval. A slightly more accurate method is the bias-corrected and accelerated bootstrap, which adjusts for bias and skewness in the bootstrap sample.

When testing for mediation, the coefficients for paths a and b are estimated in the resampled data sets and the product of the coefficients a and b (ab) is recorded. In order to estimate the indirect effect (ab) it is recommended to repeat the resampling process is at least 5000 times. The product (ab) is then rank ordered, accordingly the limits within which 95% of them fall can be discerned; this is the percentile-based bootstrap confidence interval. If zero does not fall within the upper and lower limits of the confidence interval then one can claim 95% confidence that the indirect effect is not zero.

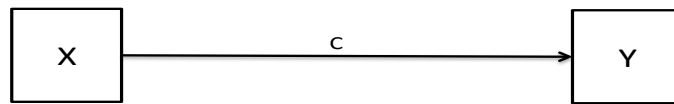


Figure 10: Diagram of a simple model of the total effect of X on Y
Note. X independent variable, Y, dependent variable

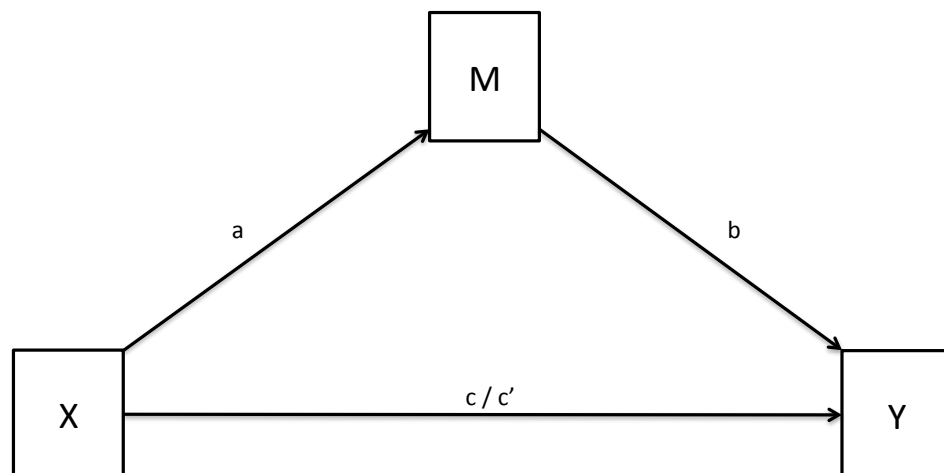


Figure 11: Diagram of a conceptual model for mediation
Note. X independent variable, Y, dependent variable, M mediator variable

Chapter 3 RESULTS

3.1 Characteristics of the sample

3.1.1 Study subjects

The sample consists of a total of 82 subjects, 46 healthy controls and 36 cases with MDD in pregnancy. Of the 36 cases, 26 (72%) met DSM-IV criteria for MDD at baseline (25 weeks gestation) and 10 (28%) were assessed, retrospectively at baseline, to have met criteria for MDD in pregnancy prior to, but not at, baseline. No subjects were taking antidepressant medication at baseline.

3.1.2 Socio-demographic factors

Socio-demographic characteristics of the participants are presented in Table 7 and the fathers of the unborn children in Table 8. There were a number of statistically significant group differences. Compared with the control group, the group with MDD in pregnancy had a greater proportion of subjects of black and minority ethnic group (21.7% v 52.8%, $\chi^2_{(1)} = 8.5$, $p = .004$, OR = 4.0, 95% CI [1.5, 10.5]), without A level or higher qualification (8.7% v 38.9%, $\chi^2_{(1)} = 10.7$, $p = .001$, OR = 6.7, 95% CI [2.0, 22.7]), not working outside the home (26.1% v 55.6%, $\chi^2_{(1)} = 7.4$, $p = .007$, OR = 3.5, 95% CI [1.4, 9.0]), not of professional or managerial employment classification (26.1% and 58.8%, $\chi^2_{(1)} = 8.7$, $p = .003$, OR = 4.0, 95% CI [1.6, 10.4]), living alone (15.2% v 52.8%, $\chi^2_{(1)} = 13.2$, $p < .001$, OR = 6.2, 95% CI [2.2, 17.6]) and whose partner was of black and minority ethnic group (23.8% v 56.5%, $\chi^2_{(1)} = 4.8$, $p = .027$, OR = 4.2, 95% CI [1.1, 15.2]). However, there were no statistically significant group differences in maternal age, maternal premorbid IQ, nor in their partner's employment status or classification of employment.

Table 7: Characteristics of the sample: maternal socio-demographic factors

	Control (n = 46)	Case (n = 34 - 36)	Statistical test and significance
Age in years at baseline, mean (SD)			
	32.6 (4.3)	30.9 (6.3)	$t_{(59.4)} = 1.4, p = .16$
Maternal ethnicity, % (n)			
White	78.3 (36)	47.2 (17)	$\chi^2_{(1)} = 8.5, p = .004$
Black and minority ethnic	21.7 (10)	52.8 (19)	
Maternal education, % (n)			
GCSE or lower	8.7 (4)	38.9 (14)	$\chi^2_{(1)} = 10.7, p = .001$
A level or higher	91.3 (42)	61.1 (22)	
Premorbid IQ (WTAR score), mean (SD) ¹			
	109.1 (12.8)	99.4 (15.6)	$t_{(38)} = 2.0, p = .08$
Maternal employment status at baseline, % (n)			
Working outside the home	73.9 (34)	44.4 (16)	$\chi^2_{(1)} = 7.4, p = .007$
Not working outside the home	26.1 (12)	55.6 (20)	
Maternal classification of employment, % (n)			
Professional or managerial	73.9 (34)	41.2 (14)	$\chi^2_{(1)} = 8.7, p = .003$
Not professional or managerial	26.1 (12)	58.8 (20)	
Marital status, % (n)			
Married or cohabiting	84.8 (39)	47.2 (17)	$\chi^2_{(1)} = 13.2, p < .001$
Living alone +/- partner	15.2 (7)	52.8 (19)	

¹ These data were not ascertained on the full sample (controls n = 25, cases n = 15).

Table 8: Characteristics of the sample: paternal socio-demographic factors

	Control	Case	Statistical test and
	(n = 19 - 21)	(n = 20 - 23)	significance
Paternal ethnicity, % (n)			
White	76.2 (16)	43.5 (10)	$\chi^2_{(1)} = 4.8, p = .027$
Black and minority ethnic	23.8 (5)	56.5 (13)	
Paternal employment status at baseline, % (n)			
Employed or student	100 (21)	95 (19)	$\chi^2_{(1)} = 1.1, p = .49^1$
Unemployed	0	5 (1)	
Paternal classification of employment, % (n) ²			
Professional or managerial	52.6 (10)	33.3 (5)	$\chi^2_{(1)} = 1.3, p = .26$
Not professional or managerial	47.4 (9)	66.7 (10)	

¹ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

² These data were not ascertained on the full sample (controls n= 19, cases n=15).

3.1.3 Obstetric history and obstetric risk factors

Obstetric history is presented in Table 9. Compared with controls, cases had a statistically significant greater proportion with a history of miscarriage (8.7% v 33.3%, $\chi^2_{(1)} = 7.8$, $p = .005$, OR = 5.2, 95% CI [1.5, 18.1]). However, there were no statistically significant group differences in history of having had a previous pregnancy, preterm delivery, elective termination of pregnancy, intra-uterine death, parity or age at menarche.

Obstetric risk factors are presented in Table 10. There were no statistically significant group differences in gestational age at baseline or in obstetric risk factors in the index pregnancy.

Table 9 Characteristics of the sample: obstetric history

	Control (n = 44 - 46)	Case (n = 36)	Statistical test and significance
History of previous pregnancy, % (n)			
	100 (46)	100 (36)	
History of preterm delivery, % (n)			
Yes	4.3 (2)	8.3 (3)	$\chi^2_{(1)} = 0.6, p = .65^1$
No	95.7 (44)	91.7 (33)	
History of miscarriage, % (n)			
Yes	8.7 (4)	33.3 (12)	$\chi^2_{(1)} = 7.8, p = .005$
No	91.3 (42)	66.7 (24)	
History of elective termination of pregnancy, % (n)			
Yes	21.7 (10)	38.9 (14)	$\chi^2_{(1)} = 2.9, p = .09$
No	78.3 (36)	61.1 (22)	
History of intrauterine death, % (n)			
Yes	2.2 (1)	0	$\chi^2_{(1)} = 0.8, p = 1.0^1$
No	97.8 (44)	100 (36)	
Nulliparous, % (n)			
Yes	47.8 (22)	41.7 (15)	$\chi^2_{(1)} = 0.3, p = .58$
No	52.2 (24)	58.3 (21)	
Age at menarche (years), mean (SD)²			
	13.2 (2.2)	13.1 (1.4)	$t_{(44)} = 0.2, p = .83$

¹ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

² These data were not ascertained on the full sample (controls n=21, cases n= 25).

Table 10: Characteristics of the sample: obstetric risk factors at baseline

	Control	Case	Statistical test and
	(n = 45 - 46)	(n = 35 - 36)	significance
Gestational age at baseline (weeks) by ultrasound dating, mean (SD)			
	26.14 (1.41)	26.48 (1.74)	t ₍₈₀₎ = 1.0, p = .34
Vaginal bleeding prior to baseline, % (n)			
Yes	21.7 (10)	31.4 (11)	χ ² ₍₁₎ = 1.0, p = .32
No	78.3 (36)	68.6 (24)	
Severity of bleeding, % (n)			
Spotting or slight	80.0 (8)	80.0 (8)	χ ² ₍₁₎ = 0, p = 1.0 ¹
Moderate or severe	20.0 (2)	20.0 (2)	
UTI in index pregnancy, % (n)			
Yes	20.0 (9)	20.0 (7)	χ ² ₍₁₎ = 0.0, p = 1.0
No	80.0 (36)	80.0 (28)	

¹ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

3.1.4 Physical health, medication, health indicators and health behaviours

3.1.4.1 Baseline

Health characteristics and medication are presented in Table 11. There were no statistically significant group differences in the presence of a chronic medical condition, use of regular medication, use of any steroid (regular or PRN) medication, pre-pregnancy BMI, tea, coffee or alcohol consumption at baseline or past history of cigarette smoking. However, compared with the control group, the group with MDD in pregnancy had a statistically significant larger proportion that had smoked in the index pregnancy (4.7% v 31.4%, $\chi^2_{(1)} = 10.0$, $p = .002$, OR = 9.4, 95% CI [1.9, 46.0]).

3.1.4.2 Thirty-two weeks gestation

Medication use at 32 weeks gestation is presented in Table 12. There was a statistically significant difference between cases and controls in use of antidepressants at 32 weeks gestation (0% v 12.5% respectively, $\chi^2_{(1)} = 5.8$, $p = .040$), however only 3 of the 25 cases were taking antidepressant drugs. There was no significant group difference in the use of medications other than antidepressants or in the use of any regular or PRN steroid medication at 32 weeks gestation.

Table 11: Characteristics of the sample: physical health and health behaviours

	Control	Case	Statistical test and significance
	(n = 43 - 46)	(n = 33 - 36)	
Chronic medical condition, % (n)¹			
Yes	13.0 (6)	13.9 (5)	$\chi^2_{(1)} = 0.01, p = 1.0^2$
No	87.0 (40)	86.1 (31)	
Taking regular medication (other than dietary supplement) at baseline, % (n)³			
Yes	2.2 (1)	11.1 (4)	$\chi^2_{(1)} = 2.8, p = .16^4$
No	97.8 (45)	88.9 (32)	
Taking regular or PRN steroid medication^{5 6}			
Yes	7.7 (2)	4.0 (1)	$\chi^2_{(1)} = 0.31, p = 1.0^4$
No	92.3 (24)	96.0 (24)	
Pre-pregnancy BMI, mean (SD)			
	23.7 (4.1)	25.4 (4.8)	$t_{(75)} = 1.7, p = .11$
Coffee (cups/week) at baseline, mean (SD)			
	2.9 (4.6)	2.6 (4.5)	$z = -0.6, p = .55$
Tea (cups/week) at baseline, mean (SD)			
	9.7 (9.6)	9.5 (14.5)	$z = -1.0, p = .30$
Alcohol use >2 units/week at baseline, % (n)			
Yes	2.3 (1)	2.9 (1)	$\chi^2_{(1)} = 0.0, p = 1.0^4$
No	97.7 (43)	97.1 (33)	
Cigarette smoking in index pregnancy, % (n)			
Yes	4.7 (2)	31.4 (11)	$\chi^2_{(1)} = 10.0, p = .002$
No	95.3 (41)	68.6 (24)	

¹ Asthma, eczema, hay fever, urticaria, endometriosis, polycystic ovarian syndrome.

² Fisher's exact test was applied as 1 cell had an expected cell count less than 5.

³ Steroid inhaler, non-steroid inhaler, proton pump inhibitor, antacid, homeopathic medicines.

⁴ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

⁵ These data were not ascertained on the full sample (controls n= 26, cases n=25).

⁶ Flixotide, Betnovate Fucibet

Table 12: Characteristics of the sample: medication use at 32 weeks gestation

	Control	Case	Statistical test and
	(n = 43 - 46)	(n = 25 - 30)¹	significance
Taking antidepressant medication at 32 weeks gestation, % (n)			
Yes	0	12.0 (3)	$\chi^2_{(1)} = 5.8, p = .040^2$
No	100 (46)	88.0 (22)	
Taking regular medication other than antidepressant or dietary supplement, % (n)³			
Yes	2.2 (1)	13.3 (4)	$\chi^2_{(1)} = 3.6, p = .15^2$
No	97.8 (44)	86.7 (26)	
Taking regular or PRN steroid medication⁴ at 32 weeks gestation, % (n)			
Yes	4.4 (2)	10.0 (3)	$\chi^2_{(1)} = 0.9, p = .38^2$
No	95.6 (43)	90.0 (27)	

¹ These data were not ascertained on the full sample of cases (n= 25 - 30).

² Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

³ Thyroxine, steroid inhaler, non-steroid inhaler, steroid cream, antihistamine, proton pump inhibitor or antacid.

⁴ Steroid inhaler and steroid cream

3.1.5 Mental health

3.1.5.1 Controls

At baseline, controls were free from current or lifetime history of any DSM-IV axis I diagnosis.

3.1.5.2 Cases

All cases (n = 36) had a diagnosis of MDD in pregnancy, either at baseline (25 weeks gestation) (n = 26, 72%), or prior to but not including baseline (n = 10, 28%).

3.1.5.2.1 Psychiatric DSM-IV diagnoses in pregnancy

Of the 36 cases with MDD at or prior to baseline, 34 (89%) had MDD alone and four (11%) had a contemporaneous anxiety disorder (specific phobia, generalized anxiety disorder, obsessive compulsive disorder). There were no other axis I disorders in pregnancy. Diagnostic data are presented in Figure 12.

3.1.5.2.2 Psychiatric DSM-IV diagnoses prior to pregnancy

Of the 36 cases, 19 (53%) had a past history of MDD alone, 5 (14%) had a past history of MDD and anxiety disorder but no other diagnoses, 4 (11%) had a past history of MDD and a diagnosis other than anxiety disorder, 3 (8%) had a past history of MDD, anxiety disorder and another diagnosis, 1 (3%) had a past history of anxiety disorder alone and 4 (11%) had no past history at all. The anxiety disorders included post traumatic stress disorder, panic disorder, specific phobia, social phobia and obsessive compulsive disorder. The other diagnoses included substance-related disorders (alcohol abuse, alcohol dependence, cocaine dependence, cannabis abuse, amphetamine dependence) and eating disorders (anorexia nervosa, eating disorder not otherwise specified). Diagnostic data are presented in Figure 12.

3.1.5.3 Psychiatric rating scale scores at baseline

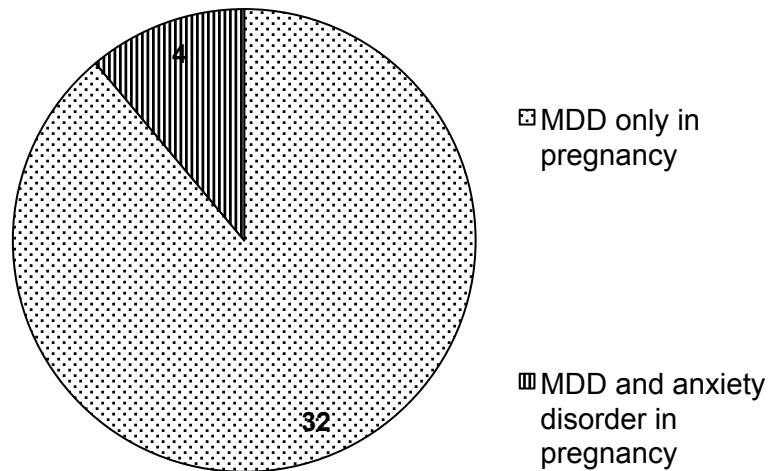
As expected, the group with MDD in pregnancy had scores demonstrating higher levels of depression symptoms with higher mean scores on BDI, this difference (-16.2, 95% CI [-20.6, -11.9]) was statistically significant and represented a large sized effect, $r = 0.80$. Furthermore, the group with MDD in pregnancy had scores demonstrating higher levels of anxiety symptoms with

higher mean scores on STAIS, this difference (-24.8, 95% CI [-29.5 -20.8]) was statistically significant and represented a large sized effect, $r = 0.81$ (Table 13).

3.1.5.4 Psychiatric rating scale scores at 32 weeks gestation

As described in the methodology, psychiatric rating scales were not completed in all subjects at 32 weeks gestation. As at baseline, the group with MDD in pregnancy had scores demonstrating higher levels of symptoms of depression with higher scores on BDI, this difference (-9.0, 95% CI [-13.6, -4.4]) was statistically significant and represented a large sized effect, $r = .60$. Furthermore, the group with MDD in pregnancy had scores demonstrating higher levels of symptoms of anxiety with higher scores on STAIS, this difference (-22.9, 95% CI [-30.1, -15.8]) was statistically significant and represented a large sized effect, $r = .79$ (Table 14).

Diagnosis in pregnancy prior to baseline



Past psychiatric history

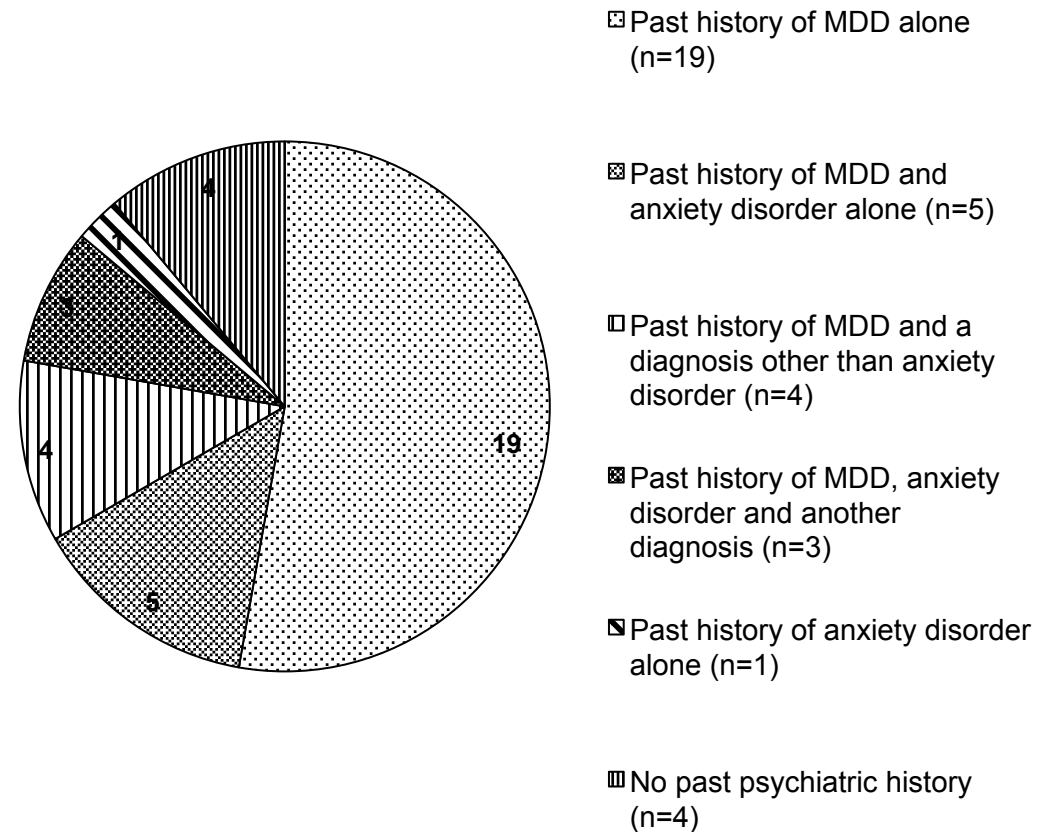


Figure 12: Cases diagnoses in pregnancy and past psychiatric history

Table 13: Characteristics of the sample: mental health at baseline

	Control	Case	Statistical test and
	(n = 44)	(n = 31)	significance
BDI, mean (SD)			
	4.0 (2.8)	20.3 (11.6)	$t_{(32.4)} = 7.6, p < .001$
STAI state, mean (SD)			
	27.3 (6.9)	52.0 (11.7)	$t_{(72.3)} = 11.7, p < .001$

Table 14: Characteristics of the sample: mental health at 32 weeks gestation

	Control	Case	Statistical test and
	(n = 18)	(n = 18)	significance
BDI, mean (SD)			
	3.7 (3.0)	12.8 (8.9)	$t = 4.0_{(28.4)}, p < .001$
STAI state, mean (SD)			
	28.0 (6.3)	51.0 (13.7)	$t = 6.6_{(25.6)}, p < .001$

3.2 Antenatal depression and maternal HPA axis in pregnancy

To test the hypotheses relating to maternal antenatal HPA axis, specifically compared to healthy pregnant women, those with antenatal depression would have higher CRH and serum cortisol and lower CRHBP at 25 weeks gestation and higher awakening, evening, diurnal cortisol secretion and cortisol awakening response at 25 and 32 weeks gestation; cases and controls were compared on these measures.

3.2.1 Maternal HPA axis at baseline

3.2.1.1 Plasma CRH and CRHBP at baseline

3.2.1.1.1 Blood sampling details

76 subjects provided a sample of plasma at baseline. Blood sampling details (gestational age at sample acquisition, time of day of sampling, interval between sample acquisition and processing and the interval between sample acquisition and CRHBP analysis) were recorded. Baseline CRH, CRHBP and blood sampling details for this sample of subjects were examined for any associations.

There was a significant positive correlation between CRH and gestational age at sample acquisition ($r_p = .31$, $p = .007$), but there was no difference in the gestational age at sample acquisition between cases and controls (weeks, M (SD), 27.7 (2.3) v 27.2 (2.3) respectively, $z = -1.3$, $p = .19$). There was no significant correlation between CRHBP and gestational age at sample acquisition, nor between CRH or CRHBP and any other blood sampling characteristics (see Appendix N, Table 48).

3.2.1.1.2 Plasma CRH, CRHBP and antenatal depression

Compared with controls, CRH at baseline was higher in cases, this difference (-15.86 ng/l, 95% CI [-37.04, 5.32]) was not statistically significant, $t_{(74)} = 1.5$, $p = .14$, and it represented a small sized effect, $r = .17$.

Compared with controls, CRHBP at baseline was higher in cases, this difference was not statistically significant and it represented a small sized effect, $r = .13$ (Table 15).

Table 15: Plasma CRH and CRHBP and antenatal depression

Control (n = 43)	Case (n = 33)	Statistical test and significance
CRH (ng/L), M (SD)		
118.18 (38.28)	134.04 (54.36)	$t_{(74)} = 1.5, p = .14$
CRHBP (pmol/L), M (SD)¹		
222.32 (136.38)	249.76 (131.63)	$z = -1.0, p = .33$

¹ Controls n = 22, cases n = 16.

3.2.1.1.3 Further analysis of CRH

Since CRH was significantly positively correlated with gestational age at sample acquisition and was higher (but not statistically significantly so) in cases, further examination was warranted. A scatter plot of CRH by gestational age at sample acquisition suggested a different relationship between these two variables in cases and in controls (Figure 13). The next step was to examine the data to see if there were any associations between CRH and other characteristics of the sample. There was a statistically significant correlation between CRH and paternal employment status at baseline and gestational age at baseline; however there was no significant difference in either of these two variables between cases and controls. There was no correlation between CRH and any other characteristics of the sample (see Appendix N, Table 49 to Table 51), thus no other factors were included as covariates in the model. Despite the absence of outliers for CRH in univariate analysis, one case was an outlier in the multivariate analysis (CRH = 307.7 ng/l, standardized residual = 3.7), however, as advised by Stevens (Stevens, 2012), it was not removed from the analysis since the Cook's distance was <1 (0.2). Furthermore, there was no evidence of other outliers or influential cases in the model. PROCESS was used to test if gestational age at sample acquisition was moderating the relationship between caseness and CRH. The model was significant, $R^2 = .18$, $F_{(3, 72)} = 3.8$, $p = .013$ (see Table 16)¹. This finding was followed up with simple slopes analysis; the conditional effect of caseness on CRH at values of gestational age at sample acquisition (mean ± 1 SD) is shown graphically in Figure 14. Further examination of this interaction using the Johnson-Neyman technique showed that when values of gestational age at sample acquisition were ≥ 28.49 weeks gestation, CRH was significantly higher in cases than in controls, and the size of effect of caseness on CRH increased as gestational age at sample acquisition got higher (see Figure 13). This finding suggests that, in this study (where gestational age at sample acquisition was simply an artefact of the study design), CRH was generally measured at a gestational age too low to show an effect of caseness, but indicates that a significant group difference in CRH (cases > controls) existed when samples were acquired, serendipitously, at later gestational ages.

¹ In order to ensure that the possible outlier did not bias this analysis, the data were winsorized; the statistically significant interaction remained.

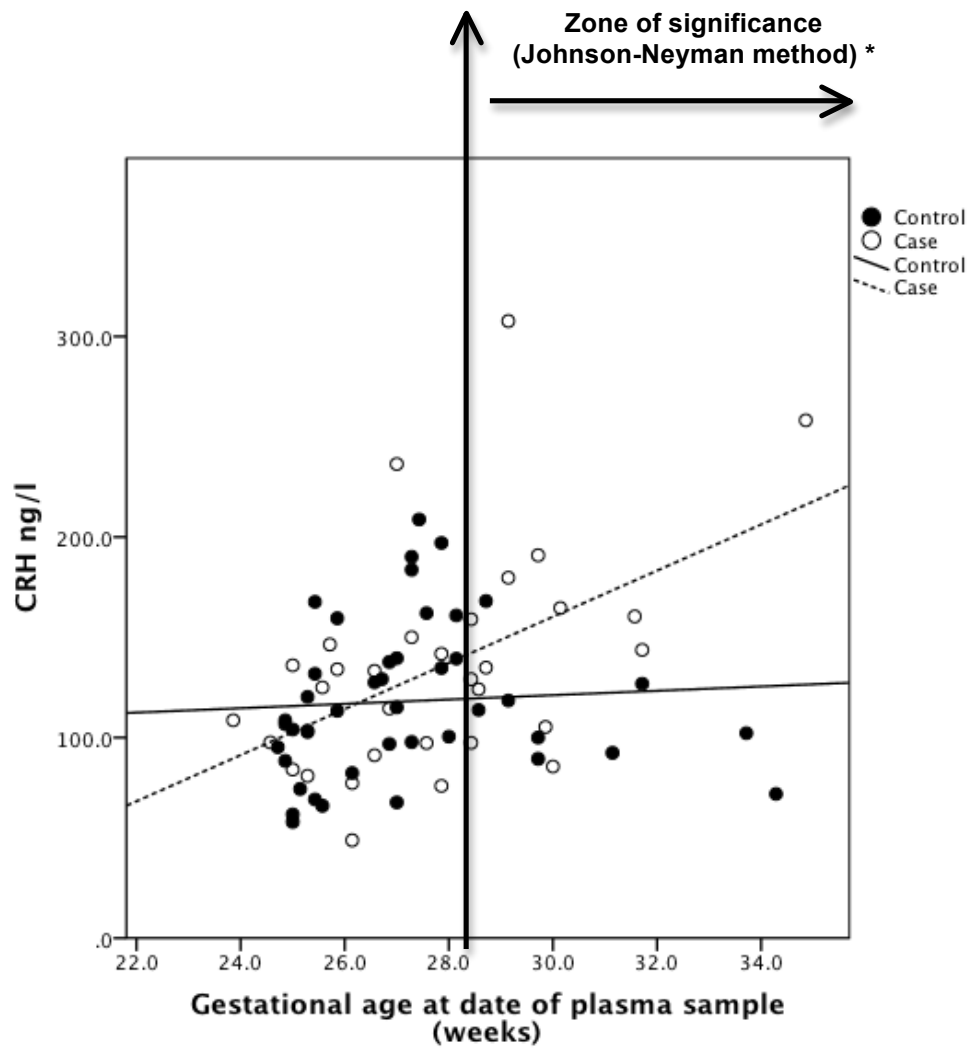


Figure 13: CRH and gestational age at sample acquisition by caseness

Note. * When values of gestational age at sample acquisition were ≥ 28.49 weeks gestation, CRH was significantly higher in cases than in controls

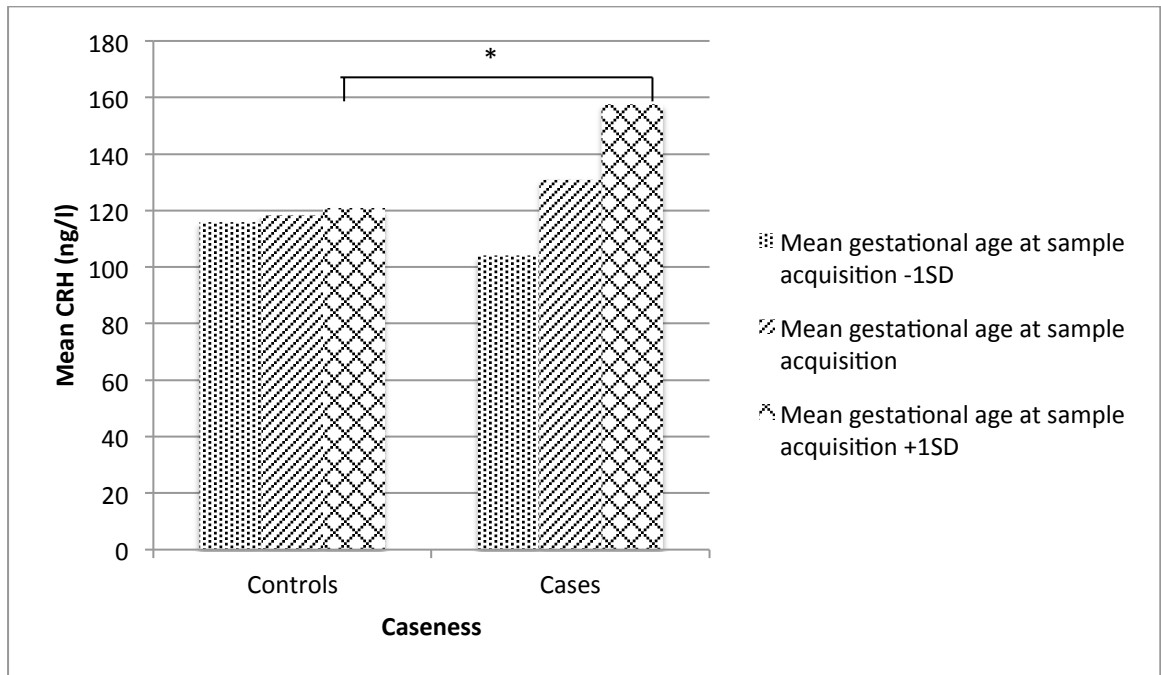


Figure 14: Moderation of CRH
 Note. *p = .029

Table 16: Linear model of predictors of CRH.

	B	SE B	t	p
Constant				
	123.8 [113.9, 133.8]	5.0	24.8	< .001
Gestational age at sample acquisition				
	5.6 [1.1, 10.1]	2.2	2.5	.014
Caseness				
	12.5 [-7.9, 33.0]	10.3	1.2	.23
Caseness x Gestational age at sample acquisition				
	10.4 [1.4, 19.4]	4.5	2.3	.024

Note. N = 76, $R^2 = .18$, bias-corrected and accelerated 95% confidence intervals are reported in parentheses, the number of bootstrap samples was set to 1000.

3.2.1.2 Serum cortisol at baseline

3.2.1.2.1 Blood sampling details

Serum cortisol was measured in 42 subjects at baseline. Blood sampling details (gestational age at sample acquisition, time of day of sampling and the interval between sample acquisition and processing) were recorded. Serum cortisol and blood sampling details for this sample of subjects were examined for any associations.

There was a significant negative correlation between serum cortisol and gestational age at sample acquisition ($r_p = -.32$, $p = .041$), but there was no difference in the gestational age at sample acquisition between cases and controls (weeks, $M (SD)$ 27.9 (2.4) v 28.1 (2.7), $t_{(40)} = 0.2$, $p = .84$). There was no significant correlation between serum cortisol and any other blood sampling characteristics (see Appendix N, Table 52).

3.2.1.2.2 Serum cortisol and antenatal depression

Compared with controls, serum cortisol at baseline was higher in cases, this difference (-34.72 nmol/l, 95% CI [-106.0, 36.5]) was not statistically significant, $t_{(40)} = 1.0$, $p = .33$, and it represented a small sized effect, $r = .16$ (Table 17).

3.2.1.2.3 Further analysis of serum cortisol

Since serum cortisol was significantly correlated with gestational age at sample acquisition and was higher (but not statistically significantly so) in cases, further examination (as for CRH in section 3.2.1.1.3 above) was warranted. In this instance, there was no moderation of the effect of caseness on serum cortisol by gestational age at sample acquisition.

Table 17: Serum cortisol and antenatal depression

	Control	Case	Statistical test and
	(n = 19)	(n = 23)	significance
Serum cortisol (nmol/L), M (SD)			
	429.84 (97.04)	464.56 (125.74)	$t_{(40)} = 1.0, p = .33$

3.2.1.3 Saliva cortisol

Data for maternal saliva cortisol are presented in the same way throughout this chapter. Firstly, data are presented for awakening and then evening cortisol since I have the largest samples for these measures at each time point throughout the study. Subsequently, data are presented for diurnal cortisol secretion (AUC) and lastly CAR (AUCi) and post-awakening cortisol (AUCg) since, as described in the methodology, there are data on a smaller subset of subjects. As described in the methodology saliva cortisol measures are not available for all subjects at baseline (see section 2.1) and samples were only used in the analyses if they were taken within the acceptable time frames described in section 2.4.2.3.4.1.

3.2.1.4 Awakening saliva cortisol at baseline

3.2.1.4.1 Saliva sampling details

39 subjects provided data for awakening cortisol at baseline. Saliva sampling details (gestational age at sample acquisition, awakening and sample time and the interval between sample acquisition and freezing) were recorded. Awakening cortisol and saliva sampling details for this sample of subjects were examined for any associations.

Awakening cortisol was significantly positively correlated with the interval between awakening and the awakening sample time at baseline ($r_s = .35$, $p = .031$), but there was no difference in this interval between cases and controls (minutes, M (SD) 2.8 (4.2) v 1.4 (3.5) respectively, $z = -1.4$, $p = .16$). There was no significant correlation between awakening cortisol and any other saliva sampling details at baseline (see Appendix N, Table 53).

3.2.1.4.2 Awakening cortisol at baseline and antenatal depression

Compared with controls, awakening cortisol at baseline was higher in cases, this difference was not statistically significant and it represented a small sized effect, $r = .10$ (Table 18).

Table 18: Awakening saliva cortisol at baseline and antenatal depression

	Control	Case	Statistical test and
	(n = 20)	(n = 19)	significance
Awakening saliva cortisol (nmol/ml), M (SD)			
	10.54 (4.82)	12.71 (5.96)	z = -0.6, p = .55

3.2.1.5 Evening saliva cortisol at baseline

3.2.1.5.1 Saliva sampling details

38 subjects provided data for evening saliva cortisol at baseline. Saliva sampling details (gestational age at sample acquisition, sample time and the interval between sample acquisition and freezing) were recorded. Evening cortisol and saliva sampling details for this sample of subjects were examined for any associations. There was no significant correlation between evening saliva cortisol at baseline and any saliva sampling characteristics (see Appendix N, Table 54).

3.2.1.5.2 Evening saliva cortisol at baseline and antenatal depression

Compared with controls, evening cortisol (natural log transformed for the analysis) at baseline was higher in cases, this difference (-0.8 nmol/ml, 95% CI [-2.1, 0.4]) was not statistically significant, $t_{(25.0)} = 1.0$, $p = .33$, and it represented a small sized effect, $r = .19$ (Table 19).

Table 19: Evening saliva cortisol at baseline and antenatal depression

	Control	Case	Statistical test and
	(n = 19)	(n = 19)	significance
Evening saliva cortisol (nmol/ml), M (SD)			
	2.52 (0.64)	3.37 (2.46)	$t_{(25.0)} = 1.0, p = .33$

3.2.1.6 Diurnal cortisol secretion at baseline

3.2.1.6.1 Saliva sampling details

35 subjects provided data for measurement of diurnal cortisol secretion at baseline. Saliva sampling details (gestational age at sample acquisition, awakening time and the interval between sample acquisition and freezing) were recorded. Diurnal cortisol and saliva sampling details for this sample of subjects were examined for any associations.

Diurnal cortisol secretion was significantly negatively correlated with time of awakening at baseline ($r_p = -.38$, $p = .04$), but there was no difference in time of awakening between cases and controls (hours, M (SD) 07:37 (0:56) v 08:04 (1:12) respectively, $t_{(33)} = 1.2$, $p = .24$). There was no significant correlation between diurnal cortisol secretion and any other saliva sampling characteristics (see Appendix N, Table 55).

3.2.1.6.2 Diurnal cortisol secretion at baseline and antenatal depression

Compared with controls, diurnal cortisol at baseline was higher in cases; this difference was not statistically significant and it represented a small sized effect, $r = .17$ (Table 20).

Table 20: Diurnal cortisol secretion at baseline and antenatal depression

	Control	Case	Statistical test
	(n = 19)	(n = 16)	and significance
Diurnal cortisol secretion (mmol/mi²), M (SD)			
	4823.4 (1931.2)	5959.3 (2183.2)	z = -1.0, p = .35

3.2.1.7 Cortisol awakening response and post-awakening cortisol at baseline

3.2.1.7.1 Saliva sampling details

21 subjects provided data for cortisol awakening response at baseline. Saliva sampling details (gestational age at sample acquisition, awakening time and the interval between sample acquisition and freezing) were recorded. CAR and post-awakening cortisol and saliva sampling details for this sample of subjects were examined for any associations.

AUC_G was negatively correlated with the time of awakening ($r_p = -.50$, $p = .026$), but there was no difference in the awakening time between cases and controls (hours, M (SD), 08:16 (1:15) v 07:31 (0:56) respectively, $t_{(19)} = 1.5$, $p = .15$). There was no significant correlation between AUC_G and any other saliva sampling characteristics. There was no significant correlation between AUC_I and any saliva sampling characteristics (see Appendix N, Table 56)

3.2.1.7.2 Cortisol awakening response and post-awakening cortisol at baseline and antenatal depression

Compared with controls, CAR (AUC_I) at baseline was lower in cases, this difference was not statistically significant, however it represented a small to medium sized effect, $r = .20$ (Figure 15). Compared with controls, post-awakening cortisol (AUC_G) at baseline was higher in cases, this difference (-16.7 nmol/ml^2 , 95% CI $[-353.1, 319.8]$) was not statistically significant, $t_{(19)} = 0.1$, $p = .92$, and it represented a negligible sized effect, $r = .02$ (Figure 16). See Table 21.

Table 21: Cortisol awakening response and post-awakening cortisol at baseline and antenatal depression

	Control	Case	Statistical test and
	(n = 12)	(n = 9)	significance
AUCi (nmol/ml²), M (SD)			
	60.4 (208.5)	40.7 (179.4)	z = -0.9, p = .42
AUCg (nmol/ml²), M (SD)			
	684.2 (361.6)	700.8 (368.4)	t ₍₁₉₎ = 0.1, p = .92

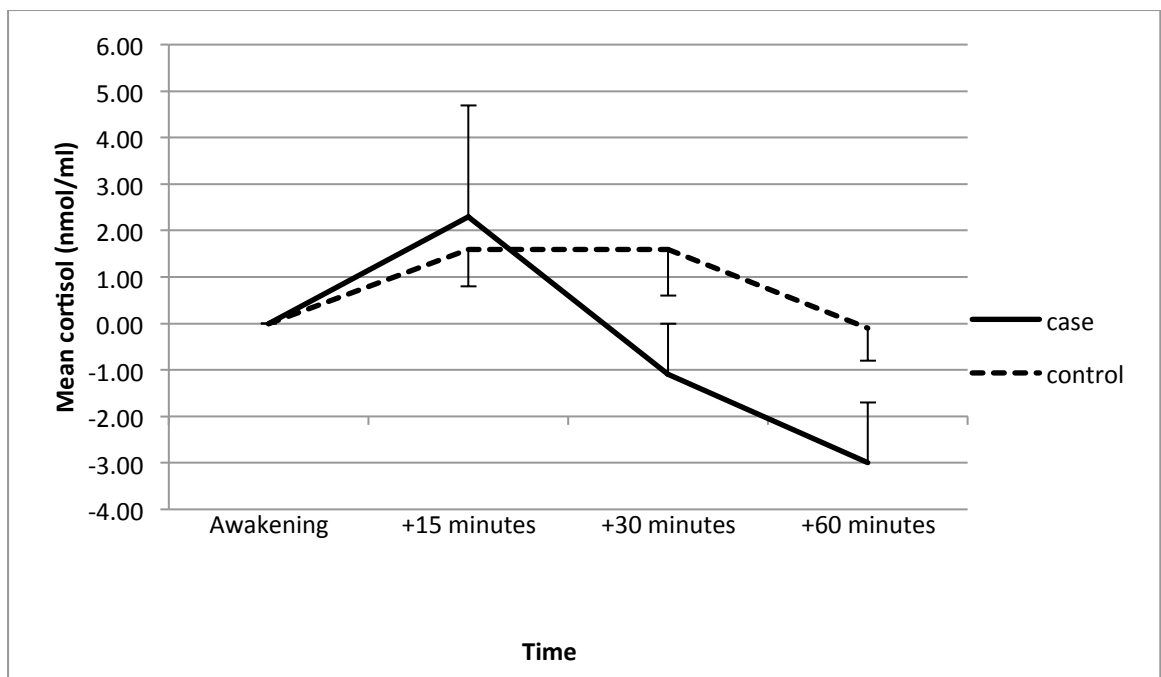


Figure 15: Cortisol awakening response (AUCi) at baseline

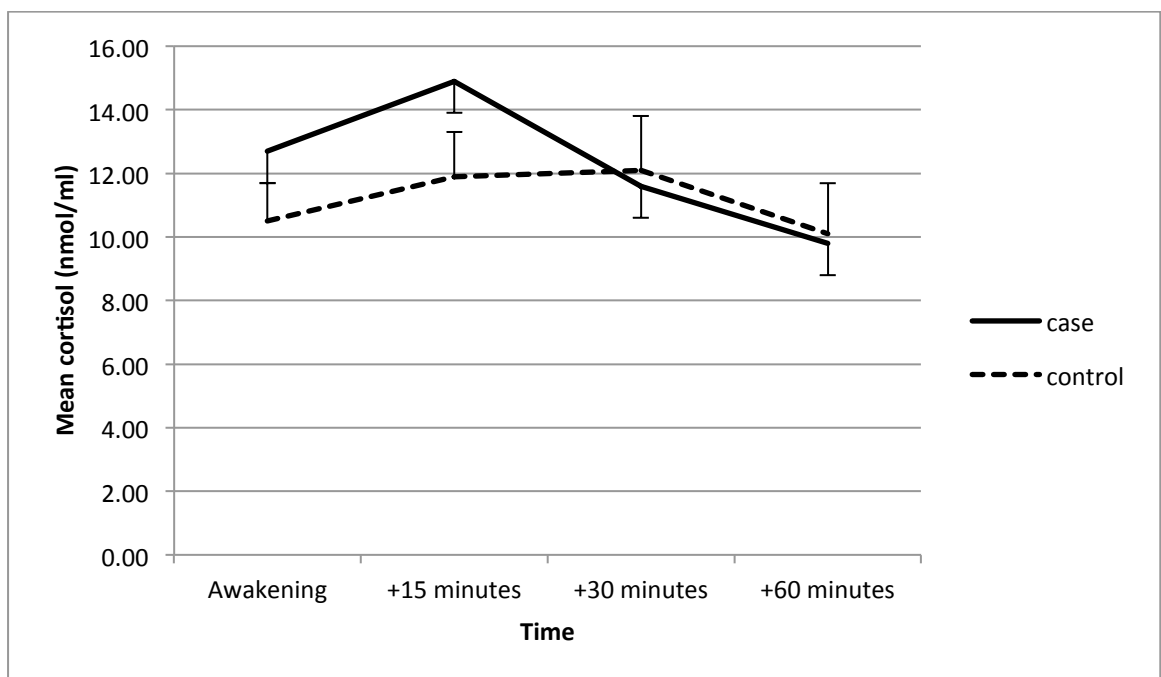


Figure 16: Post-awakening cortisol (AUCg) at baseline

3.2.2 Maternal HPA axis at 32 weeks gestation

3.2.2.1 Awakening saliva cortisol at 32 weeks gestation

3.2.2.1.1 Saliva sampling details

66 subjects provided data for awakening cortisol at 32 weeks gestation. Saliva sampling details (gestational age at sample acquisition, awakening and sample time, the interval between awakening and the sample time, and the interval between sample acquisition and freezing) were recorded. Awakening cortisol and saliva sampling details for this sample of subjects were examined for any associations. There was no significant correlation between awakening cortisol level and any saliva sampling details at 32 weeks gestation (see Appendix N, Table 57).

3.2.2.1.2 Awakening cortisol at 32 weeks gestation and antenatal depression

Cases had a higher awakening cortisol level at 32 weeks gestation compared with controls, this difference (-3.06 nmol/ml, 95% CI [-5.23, -0.89]) was statistically significant and represented a medium sized effect, $r = .33$ (Table 22).

Table 22: Awakening cortisol at 32 weeks gestation and antenatal depression

Control	Case	Statistical test and
(n = 40)	(n = 26)	significance
Awakening saliva cortisol (nmol/ml), M (SD)		
9.20 (3.77)	12.26 (5.03)	$t_{(64)} = 2.8, p = .006$

3.2.2.1.3 Awakening cortisol at 32 weeks gestation and other characteristics of the sample.

Further analyses showed that a number of other characteristics of the sample were also associated with awakening cortisol at 32 weeks gestation:

3.2.2.1.3.1 Baseline socio-demographics

Maternal age was significantly positively correlated with awakening cortisol at 32 weeks gestation ($r_p = .28$, $p = .022$); however there was no significant difference, between controls and cases, in maternal age (years, M (SD), 32.6 (4.4) v 31.7 (6.5) respectively, $t_{(39.5)} = 0.64$, $p = .56$).

Maternal ethnicity was significantly correlated with awakening cortisol at 32 weeks gestation ($r_{pb} = .28$, $p = .024$); compared with BME women ($n = 18$), the white group ($n = 48$) had significantly higher awakening cortisol (nmol/ml, M (SD), 8.36 (5.27) v 11.17 (4.02) respectively, $t_{(64)} = 2.3$, $p = .024$). Furthermore, there was a significant difference, between controls and cases, in maternal ethnicity (white, % (n), 82.5 (33) v 57.7 (15) respectively, $\chi^2_{(1)} = 4.9$, $p = .027$, OR = 3.5, 95% CI [1.2, 10.7]).

There was no significant correlation between awakening cortisol at 32 weeks gestation and any other socio-demographic factors (see Appendix N, Table 58).

3.2.2.1.3.2 Obstetric history and obstetric risk factors

Vaginal bleeding in the index pregnancy was significantly correlated with awakening cortisol at 32 weeks gestation ($r_{pb} = .29$, $p = .020$); compared with women history of vaginal bleeding in the index pregnancy ($n = 15$), those without such history ($n = 51$) had significantly higher awakening cortisol (nmol/ml, M (SD), 12.78 (4.40) v 9.70 (4.37) respectively, $t_{(64)} = 2.4$, $p = .020$). However there was no significant group difference, between controls and cases, in history of vaginal bleeding (% (n), 15.0 (6) v 34.6 (9) respectively, $\chi^2_{(1)} = 3.4$, $p = .06$).

There was no significant correlation between awakening cortisol at 32 weeks gestation and any other obstetric factors (see Appendix N, Table 59)

3.2.2.1.3.3 Physical health and medication at 32 weeks gestation and baseline health behaviours

There was no significant correlation between awakening cortisol at 32 weeks gestation and concurrent use of medication, physical health factors or baseline health behaviours or health indicators (see Appendix N, Table 60).

3.2.2.1.4 Summary of awakening cortisol at 32 weeks gestation and antenatal depression

In this sample of 66 women with cortisol data available, I found that caseness and maternal ethnicity were both associated with awakening cortisol at 32 weeks gestation. I therefore included both predictors in an analysis of variance. The model was significant ($F_{(3, 62)} = 7.3, p < .001$). There was a significant main effect of caseness on awakening cortisol ($F_{(1, 62)} = 14.0, p < .001$) independent of the effect of maternal ethnicity. Similarly there was a significant main effect of ethnicity on awakening cortisol ($F_{(1, 62)} = 12.4, p = .001$) independent of the effect of caseness. There was no interaction between ethnicity and caseness on awakening cortisol. Both white and BME women with antenatal depression had higher awakening cortisol levels compared with their non-depressed counterparts. Furthermore, white women, irrespective of antenatal depression status, had higher awakening cortisol levels compared with BME women (Figure 17).

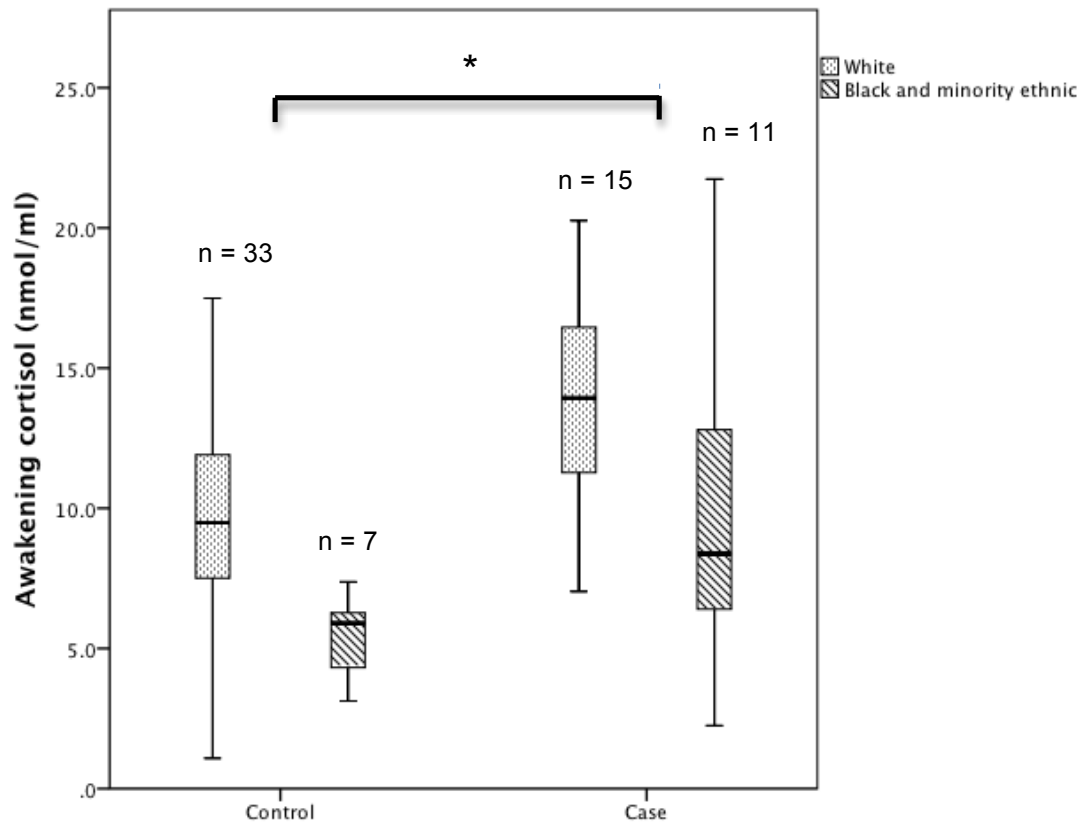


Figure 17: Awakening saliva cortisol at 32 weeks gestation, antenatal depression and maternal ethnicity

Note. ***p < .001

3.2.2.2 Evening saliva cortisol at 32 weeks gestation

3.2.2.2.1 Saliva sampling details

61 subjects provided data for evening cortisol at 32 weeks gestation. Saliva sampling details (gestational age at sample acquisition, sample time and the interval between sample acquisition and freezing) were recorded. Evening cortisol and saliva sampling details for this sample of subjects were examined for any associations.

There was a significant positive correlation between evening cortisol level and the gestational age at acquisition of the sample ($r_s = .43$, $p = .001$); however there was no significant group difference, between controls and cases, in gestational age at acquisition of the sample (weeks, M (SD), 32.6 (1.0) v 32.8 (1.4) respectively, $z = -0.6$, $p = .51$). There was no significant correlation between evening cortisol level and any other saliva sampling details (see Appendix N, Table 61).

3.2.2.2.2 Evening saliva cortisol at 32 weeks gestation and antenatal depression

The cases had a higher evening cortisol level at 32 weeks gestation compared with controls (there were a number of outliers in both groups; natural log transformation of the data corrected this source of bias and produced a normal distribution, thus the statistical analyses were performed on the transformed data). The (untransformed) difference (-5.11 nmol/ml, 95% CI [-9.65, -0.58]) was statistically significant, $t_{(59)} = 3.4$, $p = .001$, and represented a medium to large sized effect, $r = .40$ (Table 23).

Table 23: Evening saliva cortisol at 32 weeks gestation and antenatal depression

	Control	Case	Statistical test and
	(n = 38)	(n = 23)	significance
Evening saliva cortisol (nmol/ml), M (SD)			
	3.27 (3.12)	8.38 (10.26)	$t_{(59)} = 3.4, p = .001$

3.2.2.2.3 Evening cortisol at 32 weeks gestation and other characteristics of the sample

Further analyses showed that a number of other characteristics of the sample were also associated with evening cortisol at 32 weeks gestation:

3.2.2.2.3.1 Baseline socio-demographics

Employment status was significantly correlated with evening cortisol at 32 weeks gestation ($r_{pb} = .29$, $p = .025$). Compared to women working outside the home, those not working outside the home had higher evening cortisol (nmol/ml, M (SD), 3.67 (3.47) v 8.11 (10.75) respectively, $t_{(59)} = 2.3$, $p = .025$), however there was no significant group difference, between controls and cases, in women who were working outside the home (% (n), 73.7 (28) v 52.2 (12) respectively, $\chi^2_{(1)} = 2.9$, $p = .09$).

There was no significant correlation between evening cortisol level and any other socio-demographic characteristics of the sample (see Appendix N, Table 62).

3.2.2.2.3.2 Obstetric history and obstetric risk factors

Past history of miscarriage was significantly correlated with evening cortisol at 32 weeks gestation ($r_{pb} = .35$, $p = .005$). Compared to women with a past history of miscarriage, those with no such history had a lower evening cortisol (nmol/ml, M (SD), 11.42 (12.91) v 3.51 (2.96) respectively, $t_{(59)} = 2.9$, $p = .005$). Furthermore, there was a significant group difference, between controls and cases, in women with a past history of miscarriage (% (n), 10.5 (4) v 39.1 (9) respectively, $\chi^2_{(1)} = 7.0$, $p = .012$).

There was no significant correlation between evening cortisol level and any other obstetric factors (see Appendix N, Table 63)

3.2.2.2.3.3 Physical health and medication at 32 weeks gestation and baseline health behaviours

There was no significant correlation between evening cortisol at 32 weeks gestation and any physical health factors (see Appendix N, Table 64).

3.2.2.2.4 Summary of evening saliva cortisol at 32 weeks gestation and antenatal depression

In this sample of 61 women with cortisol data available, I found that caseness and a past history of miscarriage were both associated with evening cortisol at 32 weeks gestation. I therefore included both predictors in an analysis of variance. The model was significant ($F_{(3, 57)} = 6.3$, $p =$

.001). There was a significant main effect of caseness on evening cortisol ($F_{(1, 57)} = 9.3, p = .004$) independent of the effect of a history of previous miscarriage. Past history of miscarriage was no longer significant when taking account of caseness. There was no interaction between caseness and past history of miscarriage on evening cortisol. Compared with healthy controls, women with antenatal depression had a significantly higher evening cortisol level at 32 weeks gestation independent of the effect of other characteristics of the sample (Figure 18).

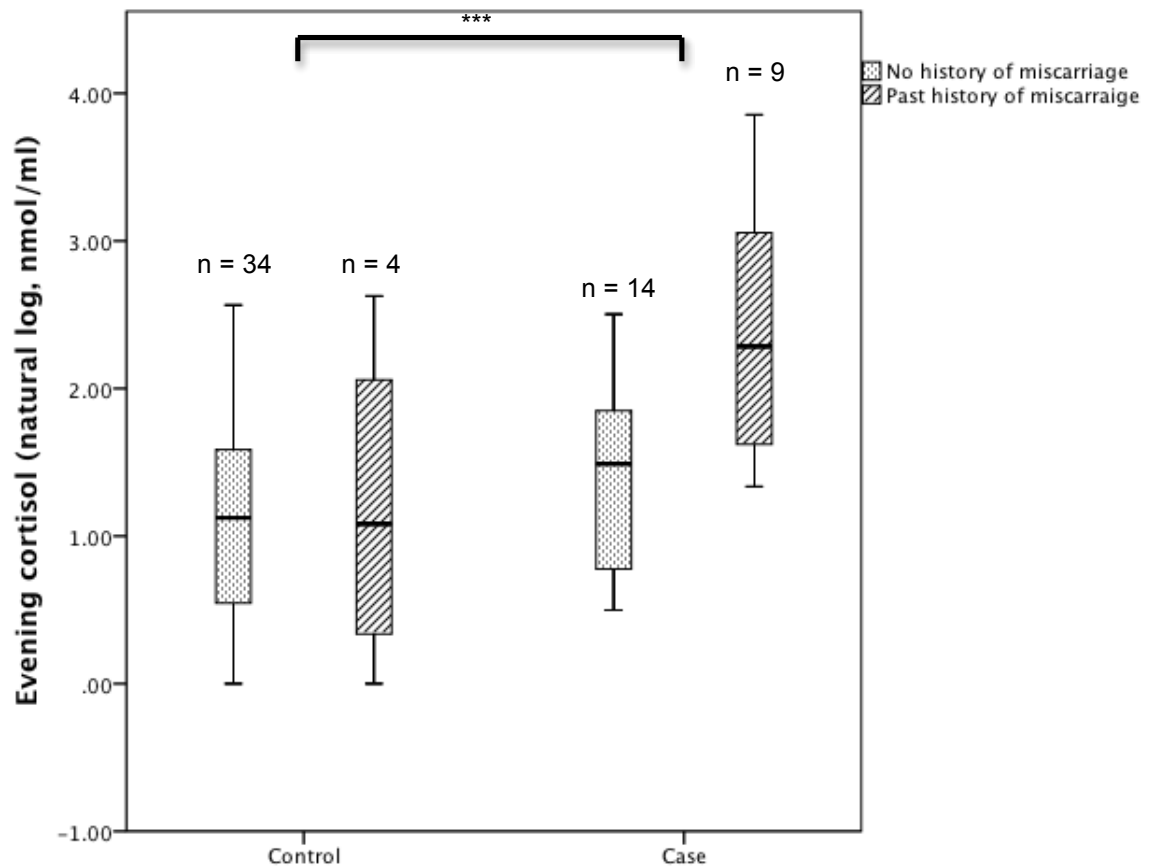


Figure 18: Evening saliva cortisol at 32 weeks gestation, antenatal depression and history of miscarriage

Note. *** $p = .001$ Evening cortisol was significantly higher in cases than controls independent of history of miscarriage.

3.2.2.3 Diurnal cortisol secretion (AUC) at 32 weeks gestation

3.2.2.3.1 Saliva sampling details

55 subjects provided both awakening and evening samples for calculation of the diurnal cortisol AUC. Saliva sampling details (gestational age at sample acquisition, awakening time and the interval between sample acquisition and freezing) were recorded. Diurnal cortisol and saliva sampling details for this sample of subjects were examined for any associations.

There was a significant positive correlation between diurnal cortisol secretion (AUC) and gestational age at sampling ($r_p = .33$, $p = .017$), however there was no significant difference in gestational age between cases and controls (weeks, M (SD), 32.9 (1.4) v 32.4 (0.6) respectively, $z = -1.1$, $p = .27$). There was no significant correlation between diurnal cortisol secretion (AUC) and any other saliva sampling details at 32 weeks gestation (see Appendix N, Table 65).

3.2.2.3.1.1 Diurnal cortisol secretion at 32 weeks gestation and antenatal depression

Diurnal cortisol secretion at 32 weeks gestation was higher in cases compared with controls (the analyses were performed on natural log transformed data), this difference ($-1878.6 \text{ nmol/ml}^2$, 95% CI $[-3151.9, -605.4]$) was statistically significant and represented a medium sized effect, $r = .38$ (Table 24).

Table 24: Diurnal cortisol secretion (AUC) at 32 weeks gestation and antenatal depression

Control	Case	Statistical test and
(n = 34)	(n = 19)	significance
Diurnal cortisol secretion (nmol/ml²), M (SD)		
4627.9 (2105.1)	6506.5 (2401.4)	t₍₅₁₎ = 2.9, p = .005

3.2.2.3.1.2 Diurnal cortisol secretion at 32 weeks gestation and other characteristics of the sample

Further analyses showed that a number of other characteristics of the sample were also associated with diurnal cortisol secretion at 32 weeks gestation:

3.2.2.3.1.2.1 Baseline socio-demographics

There was no significant correlation between diurnal cortisol secretion and any socio-demographic characteristics of the sample (see Appendix N, Table 66).

3.2.2.3.1.2.2 Obstetric history and obstetric risk factors

There was a significant positive correlation between diurnal cortisol secretion and a history of vaginal bleeding in the index pregnancy ($r_{pb} = .34$, $p = .012$); however there was no significant group difference, between controls and cases, in women who experienced vaginal bleeding (% (n), 17.6 (6) v 42.1 (8) respectively, $\chi^2_{(1)} = 3.8$, $p = .053$). There was no significant correlation between diurnal cortisol secretion at 32 weeks gestation and any other physical health factors (see Appendix N, Table 67).

3.2.2.3.1.2.3 Physical health and medication at 32 weeks gestation and baseline health behaviours

There was a significant positive correlation between diurnal cortisol secretion and coffee intake at baseline ($r_s = .30$, $p = .030$); however, there was no significant group difference, between controls and cases, in coffee intake (cups/day, M (SD), 3.2 (5.1) v 3.3 (5.0) respectively, $z = -0.1$, $p = .96$). There was no significant correlation between diurnal cortisol secretion at 32 weeks gestation and any other physical health factors (see Appendix N, Table 68).

3.2.2.3.1.3 Summary of diurnal cortisol secretion at 32 weeks gestation and antenatal depression

In this sample of 55 women with cortisol data available, I found that compared with healthy controls, women with antenatal depression had a significantly higher diurnal cortisol secretion (AUC) at 32 weeks gestation independent of the effect of other characteristics of the sample (Figure 19).

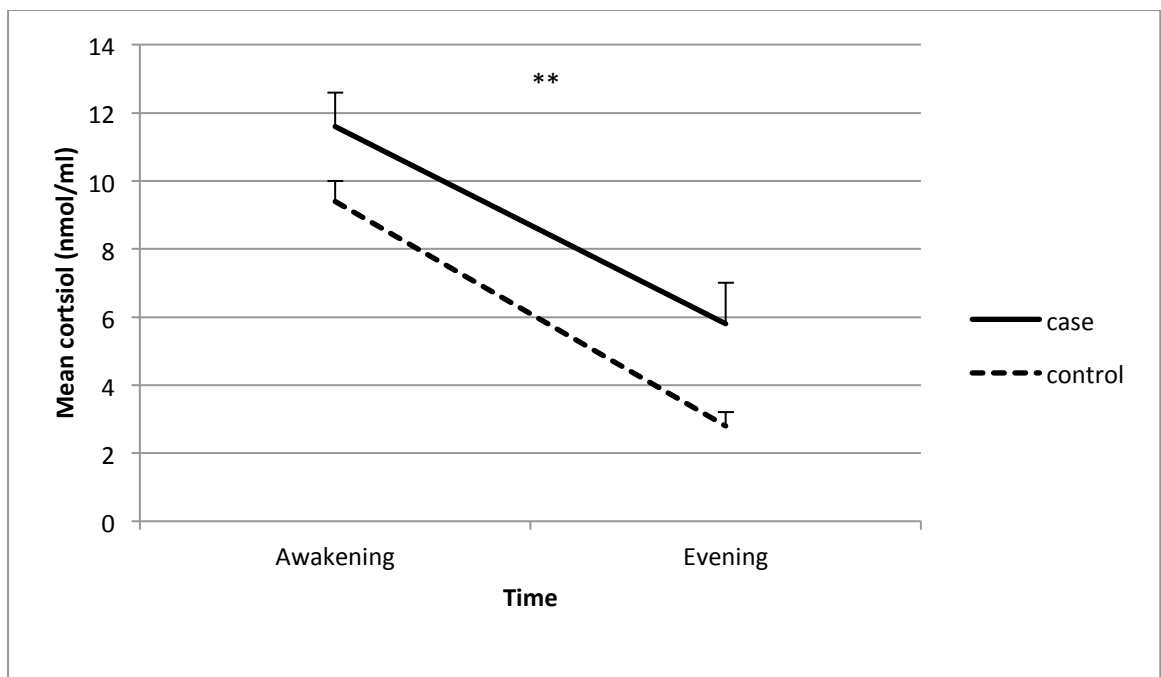


Figure 19: Diurnal cortisol secretion at 32 weeks gestation

Note. **p = .005 for AUC

3.2.2.4 Cortisol awakening response and post-awakening cortisol at 32 weeks gestation

3.2.2.4.1 Saliva sampling details

29 subjects provided data for cortisol awakening response and post-awakening cortisol at 32 weeks gestation.

Data for AUCi and AUCg were not normally distributed, but exclusion of one outlier produced a normal distribution for both variables. Data for the remaining 28 subjects are presented.

Saliva sampling details (gestational age at sample acquisition, awakening and sample time and the interval between sample acquisition and freezing) were recorded. CAR and post-awakening cortisol and saliva sampling details for this sample of subjects were examined for any associations.

There was a significant positive correlation between AUCi and the interval between sample acquisition and freezing ($r_s = .61$, $p = .002$). However, there was no significant group difference, between controls and cases, in this interval (days, M (SD), 7.5 (6.8) v 6.2 (7.5) respectively, $z = -1.1$, $p = .28$). There was no significant correlation between AUCg or AUCi and any other saliva sampling details (see Appendix N, Table 69).

3.2.2.4.2 Cortisol awakening response and post-awakening cortisol at 32 weeks gestation and antenatal depression

Cases had a lower AUCi at 32 weeks gestation compared with controls, this difference (144.7 nmol/ml², 95%CI [9.48, 279.84]) was statistically significant, $t_{(26)} = 2.2$, $p = .037$, and represented a medium to large sized effect, $r = .40$ (Figure 20 and Table 25).

Cases had a lower AUCg at 32 weeks gestation compared with controls, this difference (63.8 nmol/ml², 95%CI [9.48, 279.84]) was not statistically significant, $t_{(26)} = 0.5$, $p = .60$, and it represented a small sized effect, $r = .10$ (Figure 21 and Table 25).

Table 25 CAR and post-awakening cortisol at 32 weeks gestation and antenatal depression

	Control	Case	Statistical test and
	(n = 14)	(n = 14)	significance
AUCi (nmol/ml²), M (SD)			
	167.08 (185.10)	22.42 (162.14)	t₍₂₆₎ = 2.2, p = .037
AUCg (nmol/ml²), M (SD)			
	791.30 (354.41)	727.50 (283.30)	t₍₂₆₎ = 0.5, p = .60

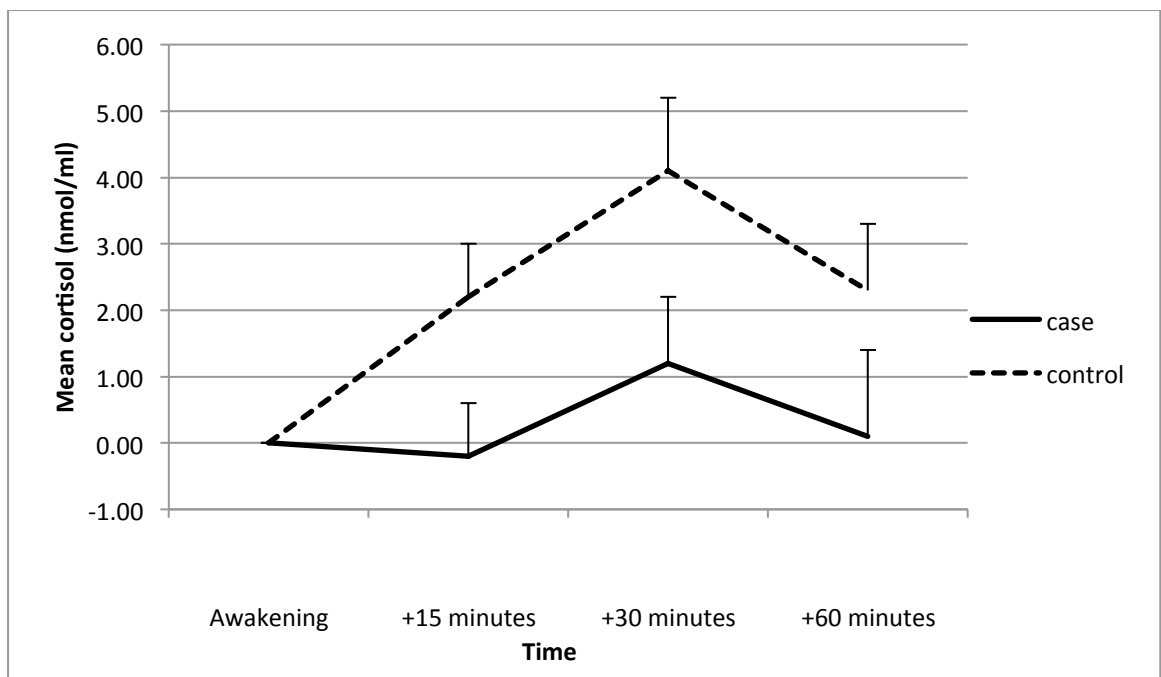


Figure 20: Cortisol awakening response (AUCi) at 32 weeks gestation

Note. $p = .037$

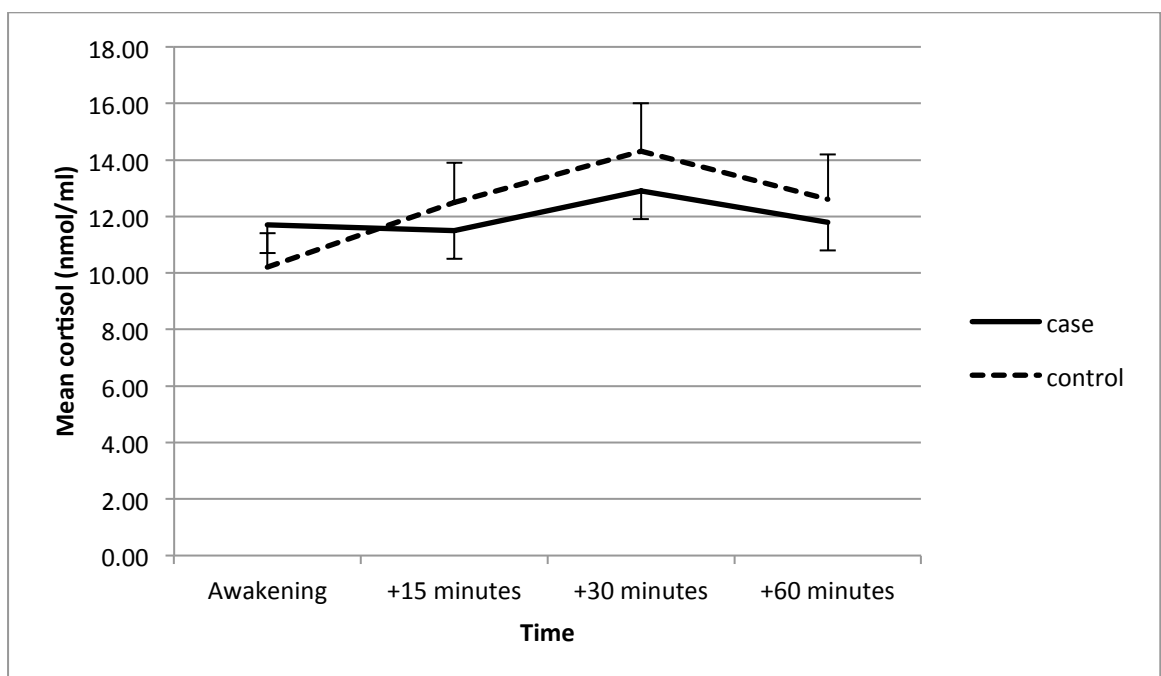


Figure 21: Post-awakening cortisol (AUCg) at 32 weeks gestation

3.2.2.4.3 Cortisol awakening response (AUCi) at 32 weeks gestation and other characteristics of the sample

There was no significant correlation between AUCi and any socio-demographic factors (see appendix Table 70), obstetric history or obstetric risk factors (see Appendix N, Table 71) or physical health and medication at 32 weeks gestation and baseline health behaviour (see Appendix N, Table 72).

3.2.2.4.4 Summary of CAR and post-awakening cortisol at 32 weeks gestation and antenatal depression

In this sample of 28 women with cortisol data available, I found that caseness was associated with AUCi at 32 weeks gestation but was not associated with AUCg. Therefore, compared with healthy controls, women with antenatal depression had a significantly lower AUCi at 32 weeks gestation independent of the effect of other characteristics of the sample.

3.2.3 Summary of maternal antenatal HPA axis and antenatal depression

Compared with healthy pregnant women, those with MDD in pregnancy had significantly higher CRH (when it was measured after 28.72 weeks gestation). Furthermore, at 32 weeks, but not at 25 weeks gestation, they had had significantly higher awakening and evening saliva cortisol, a larger diurnal cortisol secretion (AUC) and flatter cortisol awakening response (AUCi); these findings were independent of the effect of other characteristics of the sample.

3.3 Antenatal depression and obstetric outcome

To test the hypotheses that compared to healthy women those with antenatal depression would have poorer obstetric outcome; cases and controls were compared. Delivery details and obstetric outcome data were available for all subjects.

3.3.1 Delivery details

Delivery details are presented in Table 26. There were no significant differences between cases and controls in onset of labour, mode of delivery, duration of rupture of membranes, length of labour or use of analgesia in this sample.

3.3.2 Obstetric outcome

Details of obstetric outcome are presented in Table 27. Gestational age at birth for women with a spontaneous onset of labour,¹ was lower for neonates exposed to antenatal depression *in utero* compared with those not so exposed, this difference, representing 4.6 days, was statistically significant and represented a small to medium sized effect, $r = .26$.

There was no significant group difference in birth weight, head circumference at birth, the occurrence of PTB, LBW or SGA baby, Apgar scores at 1 or 5 minutes or the sex of the baby.

¹ Since there was only one preterm birth with no significant difference between cases and controls ($\chi^2_{(1)} = 0.8$, $p = 1.0$), this subject was excluded from the examination of gestational age at birth in spontaneous onset of labour.

Table 26: Delivery details and antenatal depression

	Control (n = 44 - 46)	Case (n = 33 - 36)	Statistical test and significance
Onset of labour, % (n)			
Spontaneous	73.3 (33)	76.5 (26)	$\chi^2_{(1)} = 0.1, p = .75$
Induced ¹ or no labour ²	26.7 (12)	23.6 (8)	
Type of delivery, % (n)			
Vaginal ³	76.0 (35)	77.8 (28)	$\chi^2_{(1)} = 0.0, p = .86$
Elective ⁴ or emergency ⁵ C/S	23.9 (11)	22.5 (8)	
Duration of rupture of membranes (hrs), M (SD)			
	8.26 (15.20)	4.09 (6.13)	$z = -1.1, p = .25$
Duration of labour ⁶ (hours), M (SD)			
	11.3 (10.5)	10.6 (13.0)	$z = -0.8, p = .44$
Use of analgesia ⁷ , % (n)			
None or non-pharmacological	15.4 (6)	13.3 (4)	$\chi^2_{(1)} = 0.0, p = .55^9$
Pharmacological ⁸	84.6 (33)	86.7 (26)	

Note. C/S – Caesarean Section

¹ SROM, post maturity, meconium stained liquor, IUGR, maternal infection, essential hypertension, diabetes (controls n=9, cases n=4).

² Planned Caesarean section (controls n=3, cases n=4)

³ Of the vaginal deliveries 6 were by ventouse and 2 were by forceps

⁴ Previous LSCS, ultrasound indicated a problem and failed induction of labour, maternal request, breech presentation (controls n=3, cases n=4).

⁵ Failure to progress in labour, suboptimal CTG, failed instrumentation, failed induction of labour (controls n=8, cases n=4)

⁶ Data were not ascertained on the full sample (controls n = 38, cases n = 30)

⁷ Data were not ascertained on the full sample (controls n = 39, cases n = 30)

⁸ Inhaled (controls n=19, cases n=15), IM (controls n=2, cases n=2), Epidural (controls n=12, cases n=9).

⁹ Fishers exact test was applied as 1 cell had an expected count less than 5.

Table 27: Obstetric outcome and antenatal depression

	Control	Case	Statistical test and
	(n = 46)	(n = 35-36)	significance
Gestational age at birth, M (SD) ¹			
	40.59 (1.04)	39.93 (1.08)	z = -2.0, p = .045
Birth weight (g), M (SD)			
	3513.2 (450.9)	3410.5 (511.1)	t ₍₈₀₎ = 1.0, p = .34
Preterm birth (<37 weeks gestation, % (n))			
Yes	2.2 (1)	0	$\chi^2_{(1)} = 0.8, p = 1.0^2$
No	97.8 (45)	100 (36)	
Low birth weight baby (<2500g), % (n)			
Yes	2.2 (1)	2.5 (2)	$\chi^2_{(1)} = 0.7, p = 0.58^2$
No	97.8 (45)	94.3 (33)	
Small for gestational age (<10 th centile on growth chart), % (n)			
Yes	6.5 (3)	11.1 (4)	$\chi^2_{(1)} = 0.5, p = .69^2$
No	93.5 (43)	88.9 (32)	
Head circumference at birth ³ (mm), M (SD)			
	34.7 (1.3)	34.4 (1.2)	t ₍₆₁₎ = 1.1, p = .28
Sex of the baby, % (n)			
Male	56.5 (26)	58.3 (21)	$\chi^2_{(1)} = 0.3, p = .87$
Female	43.5 (20)	41.7 (15)	
Apgar score <8 at 1 minute, % (n)			
	8.5 (1.6)	9.0 (0.6)	z = -0.5, p = .59 ²
Apgar score <9 at 5 minutes, % (n)			
	9.6 (0.7)	9.8 (0.4)	z = -1.4, p = .17 ²

¹ For spontaneous onset of labour at term, control n = 32, case n = 26

² Fishers exact test was applied as 2 cells had an expected count less than 5.

³ Data not ascertained on whole sample (controls n= 28, cases n = 20)

3.3.2.1 Gestational age at birth and characteristics of the sample

Further analyses showed that a number of characteristics of the sample were also associated with gestational age at birth in women who had a spontaneous onset of labour at term:

3.3.2.1.1 Baseline socio-demographic factors

There was a significant positive correlation between gestational age at birth and maternal age ($r_s = .26$, $p = .049$); however, there was no significant difference in maternal age between cases and controls (years, M (SD), 30.8 (6.6) v 32.3 (4.7) respectively, $z = -0.9$, $p = .37$).

There was a significant correlation between gestational age at birth and maternal ethnicity ($r_{pb} = .30$, $p = .023$); gestational age at birth was significantly lower in BME compared with white women (weeks, M (SD), 39.9 (1.1) v 40.5 (1.0) respectively, $z = -2.2$, $p = .024$). However, in contrast to the full sample described at baseline, there was no significant difference in maternal ethnicity between controls and cases (% white (n), 71.9 (23) v 50.0 (13) respectively, $\chi^2_{(1)} = 2.9$, $p = .09$).

There was a significant correlation between gestational age at birth and employment status at baseline ($r_{pb} = .26$, $p = .046$); gestational age at birth was significantly lower in women who were not working outside the home compared with women who were working outside the home (weeks, M (SD), 40.0 (1.0) v 40.5 (1.1) respectively, $z = -2.0$, $p = .047$). Furthermore, there was a significant difference in employment status between controls and cases (working outside the home, % (n), 71.9 (23) v 42.3 (11) respectively, $\chi^2_{(1)} = 5.2$, $p = .023$).

There was a significant correlation between gestational age at birth and marital status ($r_{pb} = .29$, $p = .029$); gestational age at birth was significantly lower in women living alone compared with women who were married or cohabiting (weeks, M (SD), 39.9 (1.1) v 40.5 (1.0) respectively, $z = -2.2$, $p = .031$). Furthermore, there was a significant difference in marital status between controls and cases (% living alone (n), 21.9 (7) v 57.7 (15) respectively, $\chi^2_{(1)} = 7.8$, $p = .005$).

There was no significant correlation between gestational age at delivery and any other socio-demographic factors (see Appendix O, Table 73).

3.3.2.1.2 Obstetric history and obstetric risk factors at baseline

There was no significant correlation between gestational age at birth and any baseline obstetric factors (see Appendix O, Table 74).

3.3.2.1.3 Physical health, medication, health behaviours and health indicators at baseline.

There was a significant positive correlation between gestational age at birth and pre-pregnancy BMI ($r_s = .27$, $p = .048$); however there was no significant difference in pre-pregnancy BMI between controls and cases (M (SD), 23.4 (4.4) v 24.6 (4.7) respectively, $z = -1.1$, $p = .26$).

There was a significant correlation between gestational age at birth and use of antidepressant medication at 32 weeks gestation ($r_{pb} = .31$, $p = .028$); gestational age at birth was significantly lower in women taking antidepressant medication at 32 weeks gestation compared to women who were not (weeks, M (SD), 39.0 (1.0) v 40.4 (1.1) respectively, $t_{(48)} = 2.3$, $p = .028$). Furthermore, there was a significant difference in use of antidepressant drugs at 32 weeks gestation between controls and cases (% (n), 0 v 16.7 (3) respectively, $\chi^2_{(1)} = 5.7$, $p = .042^1$).

There was no significant difference between gestational age at birth and any other antenatal physical health factors, medication use, health behaviours and health indicators (see Appendix O, Table 75).

3.3.2.2 Summary of gestational age at birth

In summary, in this sample of 58 women with spontaneous onset of labour, I found that caseness was associated with gestational age at birth. Furthermore, I found that employment status, marital status, and antidepressant use at 32 weeks gestation were also associated with both gestational age at birth and caseness. Further analysis of the use of antidepressant drugs at 32 weeks gestation was precluded since there were only three cases.

As caseness, maternal employment status and marital status were significantly associated with gestational age at birth for spontaneous deliveries at term, I included these predictors in an analysis of variance. Because having a partner and working outside the home were highly positively associated ($r\phi = .48$, $p < .001$), I first put marital status into the model with caseness predicting to gestational age. The model had a trend level of significance ($F_{(3, 54)} = 2.4$, $p = .075$), caseness was also a trend level of significance ($F_{(1, 54)} = 2.9$, $p = .095$), marital status was not significant and there was no significant interaction between marital status and caseness. Next I put employment status into the model with caseness and the model was significant ($F_{(3, 54)} = 3.4$, $p = .024$); caseness remained significant ($F_{(1, 54)} = 4.7$, $p = .034$) independently of employment

¹ Fishers exact test was applied as 2 cells had an expected count less than 5.

status, which was not significant, and there was no interaction between employment status and caseness.

Therefore, in women with a spontaneous onset of labour at term, gestational age at birth was significantly lower in cases compared with controls. This was not independent of marital status, but was independent of the effect of other characteristics of the sample.

3.3.3 Summary of obstetric outcomes and antenatal depression

In women with spontaneous onset of labour at term, compared with healthy women, those with MDD in pregnancy had significantly shorter length of gestation. This finding was independent of the effect of characteristics of the sample other than marital status. The potential effect of antidepressant medication was not taken into account as only three subjects were taking antidepressants. Lastly, cases and controls did not differ in rates of PTB, LBW or SGA babies, or in the birth weight of their baby.

3.4 Maternal antenatal HPA axis and obstetric outcome

To test the hypotheses of an association between maternal antenatal HPA axis and obstetric outcomes, correlations were performed, among subjects with a spontaneous onset of labour at term, between gestational age at birth and those antenatal HPA axis measures where there was a significant difference between cases and controls (CRH, controlling for gestational age at sample acquisition, and awakening, evening and diurnal cortisol and CAR (AUCi) at 32 weeks gestation). There were no significant correlations (Table 28).

Despite the absence of any significant correlation between gestational age at birth and the maternal antenatal HPA axis measures, it is theoretically possible that mediation was taking place, thus I used PROCESS to examine this phenomenon further. However, I found no support for the hypothesis that measures of the maternal antenatal HPA axis were mediating the association of caseness with gestational age at birth.

Secondly, in this subset of subjects with spontaneous onset of labour at term, using PROCESS, I found no evidence that the association between caseness and gestational age at birth was moderated by any of the antenatal HPA axis measures (CRH, controlling for gestational age at sample acquisition, awakening, evening or diurnal cortisol or CAR (AUCi) at 32 weeks gestation).

3.4.1 Summary of maternal antenatal HPA axis and obstetric outcome

There were no significant correlations between gestational age at birth and the maternal antenatal HPA axis measures that differed between cases and controls. Furthermore there was no evidence of mediation or moderation, by antenatal HPA axis measures, of the effect of caseness on this measure.

Table 28: Associations between gestational age at birth and maternal antenatal HPA axis measures

Maternal antenatal HPA axis					
	CRH controlling for gestational age at sample acquisition	Awakening cortisol at 32 weeks gestation	Evening cortisol at 32 weeks gestation	Diurnal cortisol at 32 weeks gestation	CAR (AUCi) at 32 weeks gestation
Gestational age at birth	-.06	.18	-.16	.04	-.04
n	50	46	41	35	18

Note. Spearman's correlation coefficients are presented.

3.5 Infant HPA axis at 8 weeks postnatal

3.5.1 Study subjects

At eight weeks postnatal seven (9%) subjects had dropped out of the study leaving 75 (91%) subjects, 45 (98%) controls and 30 (83%) cases.

3.5.2 Infant cortisol response to the pain of immunization at 8 weeks postnatal (day 1)

To test the hypotheses that compared with 8-week-old infants not exposed to depression *in utero*, 8-week-old infants so exposed would have a larger cortisol response to the pain of immunization; infants of cases and controls were compared. 54 infants provided saliva samples both before and 20 minutes after routine immunization at 8 weeks postnatal. The saliva sampling details and cortisol are presented followed by the characteristics of infants and their mothers.

3.5.2.1 Saliva sampling details

Cortisol levels before and 20 minutes after routine immunization and the change (delta cortisol) were measured. Saliva sampling details (time of sample before the immunization, time of the immunization, interval between the first sample and the immunization) were recorded. Infant cortisol and saliva sampling details for this sample of subjects were examined for any associations. There were no significant correlations between any of the three infant cortisol measures and any saliva sampling characteristics at 8 weeks postnatal (see Appendix P, Table 76).

3.5.2.2 Infant cortisol response to immunization at 8 weeks postnatal and maternal antenatal depression

Infants exposed to depression *in utero* were compared with those not so exposed. Cortisol after the immunization was higher in infants exposed to depression *in utero* compared with those not so exposed, this difference (-10.45 nmol/ml, 95% CI [-21.8, -.1] was at a trend level of significance, $t_{(19.9)} = 1.9$, $p = .069$, however it represented a medium to large sized effect, $r = .40$. Delta cortisol was also higher in infants exposed to depression *in utero* compared with those not so exposed, this difference (-4.93 nmol/ml, 95% CI [-11.6, 1.8], was not statistically significant, however it represented a small to medium sized effect, $r = .20$. Cortisol before the immunization was higher in infants exposed to depression *in utero* compared with those not so exposed,

however, this difference was not statistically significant and represented only a small sized effect, $r = .16$ (Table 29).

On visual inspection the change in cortisol from before to after the immunization appeared different between infants of cases and controls (Figure 22), hence further examination of the data was warranted. The Wilcoxon signed-rank test is a non-parametric test for repeated measures and it allows examination of the effect size of the change in cortisol for cases and controls separately. For both groups the cortisol was significantly higher after than before immunization; however, the effect was larger in cases than in controls. Cases, median = 6.5 before and 14.7 after the immunization, $z = -3.2$, $p = .001$, $r = -.52$, this represents a large effect for the cases group. Controls, median = 5.1 before and 10.6 after the immunization, $z = -3.2$, $p = .001$, $r = -.37$, this represents only a medium change in levels of cortisol for the control group. Thus the effect size was greater for infants who were exposed to depression *in utero* than those who were not so exposed.

Table 29: Infant cortisol response to immunization at 8 weeks postnatal and maternal antenatal depression

	Control	Case	Statistical test and
	(n = 36)	(n = 18)	significance
Cortisol before the immunization (nmol/ml), M (SD)			
	6.88 (5.38)	12.41 (19.08)	$z = -1.2, p = .25$
Cortisol 20 minutes after the immunization (nmol/ml), M (SD)			
	12.69 (9.03)	23.14 (22.18)	$t_{(19.9)} = 1.9, p = .069$
Delta cortisol (nmol/ml), M (SD)			
	5.81 (11.14)	10.74 (12.41)	$t_{(52)} = 1.5, p = .15$
Effect size for the change in cortisol from pre- to post-immunization			
	Medium effect	Large effect	
	$r = .37, p = .001$	$r = .52, p = .001$	

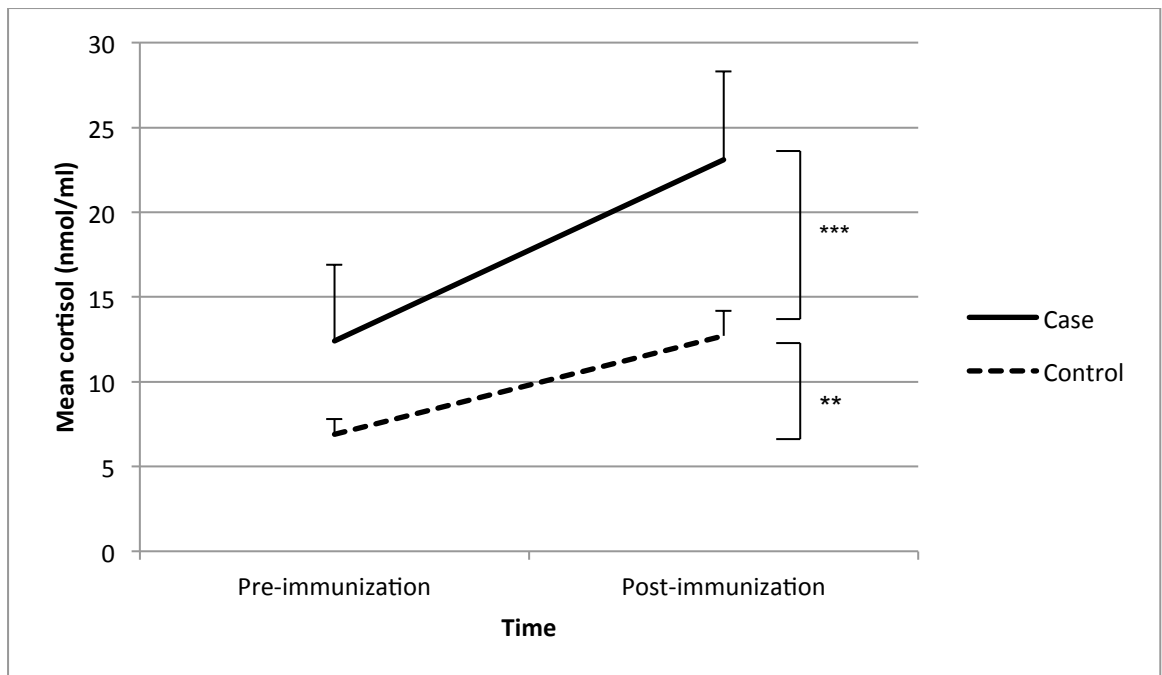


Figure 22: Infant cortisol response to immunization at 8 weeks postnatal

Note. ***Cases, large effect size ($p = .001$). **Controls, medium effect size ($p = .001$).

3.5.2.3 Infant cortisol response to immunization at 8 weeks postnatal and other infant and maternal factors

The characteristics of infants who provided saliva samples on the day of immunization at 8 weeks postnatal are presented; maternal factors are also presented since they are potential confounding factors in the analysis of infant cortisol. Infants of cases (thus exposed to antenatal depression *in utero*) and infants of controls (not exposed to antenatal depression *in utero*) were compared.

3.5.2.3.1 Infant characteristics at 8 weeks postnatal

Compared with infants of controls, a statistically significant greater proportion of infants of cases were taking regular medication at 8 weeks postnatal (0 v 16.7% respectively). There were no significant group differences in infant age at assessment, breastfeeding, health or medication use (Table 30).

3.5.2.3.2 Infant factors prior to 8 weeks postnatal

Compared with infants of controls, a greater proportion of infants of cases had been exposed to maternal smoking *in utero* (2.9% v 47.1% respectively, $\chi^2_{(1)} = 15.6$, $p < .001$, OR = 30.2, 95% CI [3.3, 274.1]) and maternal symptoms of anxiety *in utero* (STAIS, M (SD), 27.9 (7.4) v 52.5 (9.6) respectively); this difference was statistically significant, $t_{(52)} = 9.8$, $p = .001$ and represented a medium to large sized effect, $r = 0.74$. There was no significant group difference in exposure to antidepressant medication *in utero*, gestational age at birth, mode of delivery, APGAR scores at birth, the sex of the infant or any cluster scores of the NBAS examination (which was performed in the neonatal period) (Table 31).

3.5.2.3.3 Maternal characteristics at 8 weeks postnatal (Table 32)

3.5.2.3.3.1 Maternal medication and health behaviours

Compared with controls, a statistically significant greater proportion of cases were taking regular medication (2.9% v 38.9% respectively, $\chi^2_{(1)} = 12.0$, $p = .001$, OR 21.6 [2.4, 195.8]) and smoking (0% v 33.3% respectively, $\chi^2_{(1)} = 6.6$, $p = .021$) at 8 weeks postnatal. There were no significant group differences in any other maternal medication use or health behaviours at 8 weeks postnatal.

3.5.2.3.3.2 Maternal mental health

There was no significant difference between cases and controls in a current diagnosis of MDD (see Table 32); the two cases who met criteria for MDD at 8 weeks postnatal were both taking antidepressant medication at 8 weeks postnatal, however, only two other cases were taking antidepressant medication at this assessment, therefore the absence of a group difference in a current diagnosis of MDD 8 weeks postnatal was unlikely to be attributable to use of antidepressant medication. However, compared with controls, cases had a longer duration of exposure to MDD in the postnatal period (weeks, M (SD), 0 v 1.6 (2.8) respectively), this difference was statistically significant and represented a medium to large sized effect, $r = .46$.

3.5.2.3.3.3 Maternal cortisol

There was no significant group difference in any maternal cortisol measure (awakening, evening, diurnal cortisol, AUCg and AUCi) at 8 weeks postnatal.

3.5.2.3.4 Summary of infant and maternal characteristics

As described above, there were significant differences between cases and controls in a number of infant and maternal characteristics (infants taking regular medication, exposure to smoking *in utero*, exposure to symptoms of anxiety *in utero*, mothers who were taking regular medication and who were smoking at 8 weeks postnatal and maternal duration of MDD in the postnatal period). However, there were no significant correlations between the infant cortisol measures and any of these potential confounding factors (see Appendix P, Table 77); thus they were not accounting for the differences found in infant cortisol response to immunization at 8 weeks postnatal.

3.5.2.4 Summary of infant cortisol response to immunizations and antenatal depression at 8 weeks postnatal

In this sample of 54 infants with cortisol data before and 20 minutes after routine immunizations at 8 weeks postnatal, I found that compared with infants not exposed to depression *in utero*, infants who were exposed to depression *in utero* had a higher cortisol level after the immunizations. This was only at a trend level of significance, however, it represented a medium to large sized effect. Furthermore, delta cortisol was also higher in infants exposed to depression *in utero*; this difference was not statistically significant, however it represented a small to medium sized effect. Moreover, immunization was associated with an increase in cortisol equivalent to a large effect

size in cases but a smaller (medium) effect size in controls. These findings were independent of other infant or maternal factors.

Table 30: Infant characteristics at 8 weeks postnatal (day 1)

	Control	Case	Statistical test and significance
	(n = 36)	(n = 18)	
Baby's age at assessment (weeks), M (SD)			
	9.5 (1.8)	9.3 (1.8)	$t_{(52)} = 0.4, p = .71$
Receiving breast milk at 8 weeks postnatal, % (n)			
No	11.1 (4)	22.2 (4)	$\chi^2_{(1)} = 1.2, p = .42^1$
Yes	88.9 (32)	77.8 (14)	
Ever received breast milk, % (n)			
No	0	5.6 (1)	$\chi^2_{(1)} = 2.0, p = .33^2$
Yes	100 (36)	94.4 (17)	
Any feeding problems, % (n)³			
No	58.8 (10)	66.7 (8)	$\chi^2_{(1)} = 0.2, p = .72^1$
Yes	41.2 (7)	33.3 (4)	
Non-routine appointment with a doctor, % (n)³			
No	70.6 (12)	41.7 (5)	$\chi^2_{(1)} = 2.4, p = .15^1$
Yes	29.4 (5)	58.3 (7)	
Taking regular medication, % (n)⁴			
No	100 (36)	83.3 (15)	$\chi^2_{(1)} = 6.4, p = .033^2$
Yes	0	16.7 (3)	

¹ Fisher's exact test was applied as 1 cell had an expected cell count less than 5.² Fisher's exact test was applied as 2 cells had an expected cell count less than 5.³ These data were not ascertained on the full sample (controls n=17, cases n= 12).⁴ Chloramphenicol eye drops, daktarin oral gel, gaviscon, hydrocortisone cream.

Table 31: Infant factors prior to 8 weeks postnatal (day 1)

	Control (n = 36)	Case (n = 17-18)	Statistical test and significance
Exposed to antidepressants <i>in utero</i>, % (n)¹			
No	100 (36)	84.6 (11)	$\chi^2_{(1)} = 5.8, p = .07^2$
Yes	0	15.4 (2)	
Exposed to smoking <i>in utero</i>, % (n)			
No	97.1 (34)	52.9 (9)	$\chi^2_{(1)} = 15.6, p < .001$
Yes	2.9 (1)	47.1 (8)	
Exposure to symptoms of anxiety <i>in utero</i> (STAIS score), M (SD)			
	27.9 (7.4)	52.5 (9.6)	$t_{(52)} = 9.8, p = .001$
Gestational age at birth (weeks), M (SD)			
	40.31 (1.60)	40.06 (1.09)	$t_{(52)} = 0.6, p = .55$
Vaginal mode of delivery, % (n)			
No	25.0 (9)	16.7 (3)	$\chi^2_{(1)} = 0.5, p = .73^3$
Yes	75.0 (27)	83.3 (15)	
Apgar score at 1 minute, M (SD)			
	8.6 (1.7)	9.0 (0.6)	$z = 0.0, p = 1.0$
Apgar score at 5 minutes, M (SD)			
	9.6 (0.7)	9.8 (0.4)	$z = -1.2, p = .25$
Sex of the baby, % (n)			
Male	52.8 (19)	50.0 (9)	$\chi^2_{(1)} = 0.0, p = .87$
Female	52.8 (17)	50.0 (9)	
NBAS orientation cluster, median			
	7.7	6.9	$z = -1.7, p = .09$
NBAS motor cluster, median			
	5.7	5.6	$z = -1.3, p = .20$
NBAS range of state cluster, median			
	2.8	3.3	$z = -1.31, p = .20$

¹ These data were not ascertained on the full sample (cases n= 13).² Fisher's exact test was applied as 2 cells had an expected cell count less than 5³ Fisher's exact test was applied as 1 cell had an expected cell count less than 5.

NBAS regulation of state cluster, median			
	6.0	6.0	$z = -0.3, p = .79$
NBAS autonomic stability cluster, median			
	6.0	5.3	$z = -1.6, p = .11$
NBAS total number of abnormal reflexes			
	1.0 (1.9)	1.0 (1.3)	$z = -0.4, p = .68$

Table 32: Maternal characteristics at 8 weeks postnatal (day 1)

	Control (n = 35-36)	Case (n = 16-18)	Statistical test and significance
Taking regular medication at 8 weeks postnatal, % (n)¹			
No	97.1 (34)	61.1 (11)	$\chi^2_{(1)} = 12.0, p = .001^2$
Yes	2.9 (1)	39.9 (7)	
Taking regular or PRN steroid medication at 8 weeks postnatal, % (n)			
No	94.3 (33)	83.3 (15)	$\chi^2_{(1)} = 1.7, p = .32^3$
Yes	5.7 (2)	16.7 (3)	
Coffee (cups/week) at 8 weeks postnatal, mean (SD)⁴			
	4.4 (5.3)	4.3 (7.6)	$z = -1.2, p = .35$
Tea (cups/week) at 8 weeks postnatal, mean (SD)⁴			
	9.8 (13.4)	10.6 (15.7)	$z = -0.7, p = .46$
Alcohol use (units/week) at 8 weeks postnatal, % (n)⁴			
	1.5 (2.1)	0.6 (1.0)	$z = -1.0, p = .30$
Cigarette smoking at 8 weeks postnatal, % (n)⁵			
No	100 (17)	66.7 (8)	$\chi^2_{(1)} = 6.6, p = .021^3$
Yes	0	33.3 (4)	
Current diagnosis of DSM-IV MDD at 8 weeks postnatal, % (n)			
No	100 (36)	88.9 (16)	$\chi^2_{(1)} = 4.2, p = .11^3$
Yes	0	11.1 (2)	
Duration of exposure to MDD in the postnatal period (weeks), M (SD)			
	0	1.6 (2.8)	$z = -3.4, p = .002$
Awakening cortisol at 8 weeks postnatal (nmol/ml), M (SD)⁶			
	8.11 (3.45)	8.57 (4.38)	$t_{(47)} = 0.4, p = .69$
Evening cortisol at 8 weeks postnatal (nmol/ml), M (SD)⁷			
	1.45 (0.77)	1.53 (0.76)	$t_{(36)} = 0.3, p = .76$

¹ Thyroxine, OCP, clexane, beclomethosone inhaler, daktarin cream, sertraline.² Fisher's exact test was applied as 1 cell had an expected cell count less than 5.³ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.⁴ Not ascertained on full sample (controls n=17, cases n=11)⁵ Not ascertained on full sample (controls n=17, cases n=12)⁶ Awakening cortisol was not ascertained on the whole sample of controls (n = 32)⁷ Evening cortisol was not ascertained on the whole sample (controls n = 23 cases n = 15)

Diurnal cortisol (nmol/ml ²) at 8 weeks postnatal, M (SD) ¹		
3511.0 (1245.4)	3561.4 (1371.2)	$t_{(34)} = 0.1, p = .91$
Post-awakening cortisol (AUCg) (nmol/ml ²) at 8 weeks postnatal, M (SD) ²		
567.1 (229.4)	666.3 (238.2)	$t_{(16)} = 0.8, p = .42$
CAR (AUCi) (nmol/ml ²) at 8 weeks postnatal, M (SD) ²		
53.5 (185.9)	128.1(266.8)	$t_{(16)} = 0.7, p = .49$

¹ Diurnal cortisol was not ascertained on the whole sample (controls n = 21 cases n =15)

² CAR was not ascertained on the whole sample (controls n = 11 cases n = 7)

3.5.3 Associations of infant cortisol response to immunizations at 8 weeks postnatal with maternal antenatal HPA axis

To test the hypotheses that infant HPA axis function at 8 weeks postnatal would be associated with maternal antenatal HPA axis, correlations were performed to examine associations between the infant cortisol measures at 8 weeks postnatal and the maternal antenatal HPA axis measures that differed between cases and controls (CRH, controlling for gestational age at sample acquisition and awakening, evening and diurnal cortisol and CAR (AUCi) at 32 weeks gestation). There were no significant associations between the infant cortisol and maternal HPA axis measures (Table 33).

Despite the absence of any significant correlation between these maternal antenatal HPA axis measures and infant cortisol response to the pain of immunization, it is theoretically possible that mediation was taking place, thus I used PROCESS to examine this phenomenon further. However, I found no support for the hypothesis that measures of the maternal antenatal HPA axis were mediating the association of caseness with infant cortisol response to immunizations at 8 weeks postnatal.

Using PROCESS, I examined if the infant cortisol measures were moderated by any of the maternal antenatal cortisol measures that differed between cases and controls. I found that infant delta cortisol was moderated by the interaction of caseness and maternal diurnal cortisol (AUC) at 32 weeks gestation. No other variables were associated with the infant or maternal cortisol measures thus no other covariates were included in the model. The model was significant, $R^2 = .22$, $F_{(3, 32)} = 3.0$, $p = .045$ (see Table 34). This finding was followed up with simple slopes analysis; the conditional effect of caseness on delta cortisol at values of maternal antenatal diurnal cortisol is shown graphically in Figure 23; when the value of maternal antenatal diurnal cortisol was +1SD above the mean (7648.2 nmol/ml) delta cortisol was statistically significantly higher in infants exposed to depression *in utero* compared with infants not so exposed. Further examination of this interaction using the Johnson-Neyman technique showed that the effect of caseness on delta cortisol became statistically significant when values of maternal antenatal diurnal cortisol were ≥ 6938.6 nmol/ml² and this effect became greater with increasing levels of maternal antenatal diurnal cortisol (see Figure 24). Putting this into context, as described in

section 3.2.2.3.1.3 above, maternal antenatal diurnal cortisol at 32 weeks gestation was higher in cases than controls.

There were no other moderating effects of maternal antenatal cortisol on the association between caseness and infant cortisol at 8 weeks postnatal.

Table 33: Infant cortisol response to immunizations at 8 weeks postnatal and maternal antenatal HPA axis

Maternal antenatal HPA axis					
	CRH controlling for gestational age at sample acquisition	Awakening cortisol at 32 weeks gestation	Evening cortisol at 32 weeks gestation	Diurnal cortisol at 32 weeks gestation	CAR (AUCi) at 32 weeks gestation
Pre	-.04	.01	-.27	.00	-.19
Post	.10	.08	-.09	.06	-.16
Delta	.13	.19	.03	.20	-.16
n	47	42	42	36	21

Note. Pearson's *r* coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis.

Pre = cortisol before the immunizations

Post = cortisol 20 minutes after the immunizations

Delta = change in cortisol from before to after the immunizations

3.5.3.1 Summary of the associations of infant cortisol response to immunizations at 8 weeks postnatal with maternal antenatal HPA axis

There were no significant correlations between cortisol response to immunization in 8-week-old infants and the maternal antenatal HPA axis measures that differed between cases and controls. Furthermore, there was no evidence of mediation of the effect of caseness on these infant measures. However, the change in cortisol from before to after the immunization (delta cortisol) was moderated by the interaction of caseness and maternal antenatal diurnal cortisol at 32 weeks gestation such that infants exposed to depression *in utero* had a larger delta cortisol at 8 weeks postnatal when maternal antenatal diurnal cortisol was high. Furthermore, as described above, maternal antenatal diurnal cortisol at 32 weeks gestation was higher in cases than controls, indicating a continuity of overactivity of both maternal antenatal HPA axis and infant HPA axis.

Table 34: Linear model of predictors of infant delta cortisol

	B	SE B	t	p
Constant				
	6.2 [2.7, 9.6]	1.7	3.6	< .001
Maternal antenatal diurnal cortisol				
	0.001 [-0.001, 0.003]	0.001	1.1	.27
Caseness				
	0.2 [-7.7, 8.0]	3.8	0.04	.96
Interaction (caseness x diurnal cortisol)				
	0.005 [0.001, 0.009]	0.002	2.8	.009

Note. N = 54, $R^2 = .22$, bias-corrected and accelerated 95% confidence intervals are reported in parentheses, the number of bootstrap samples was set to 1000.

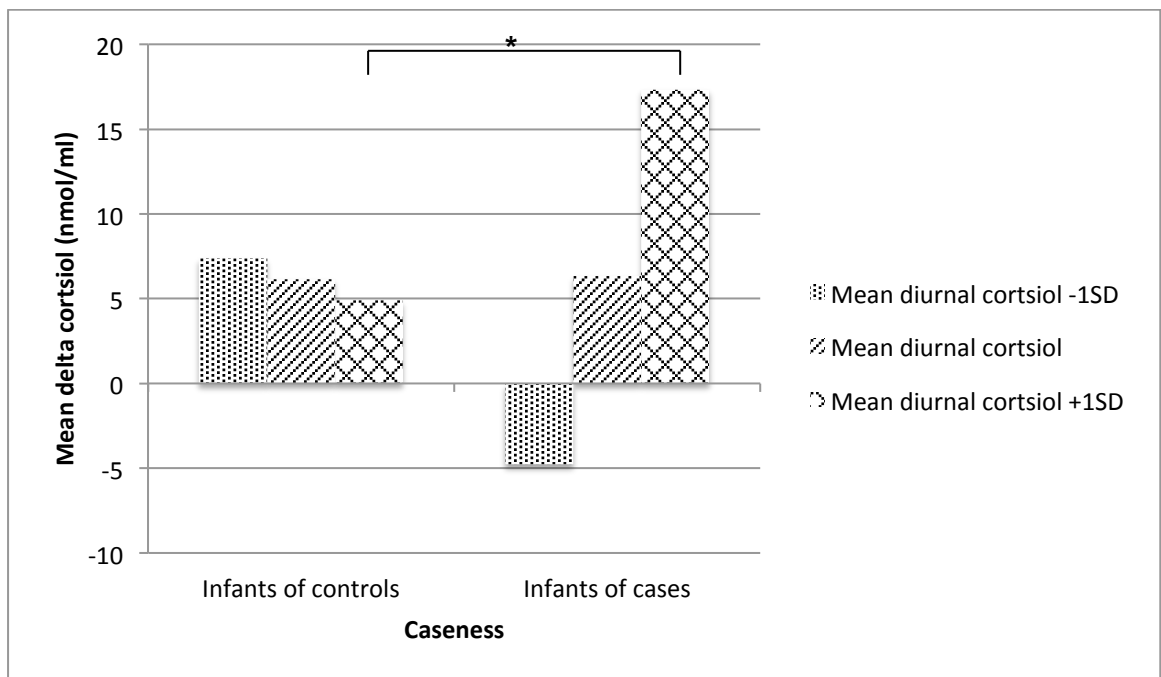


Figure 23: Moderation of infant delta cortisol at 8 week postnatal

Note. *p= .020

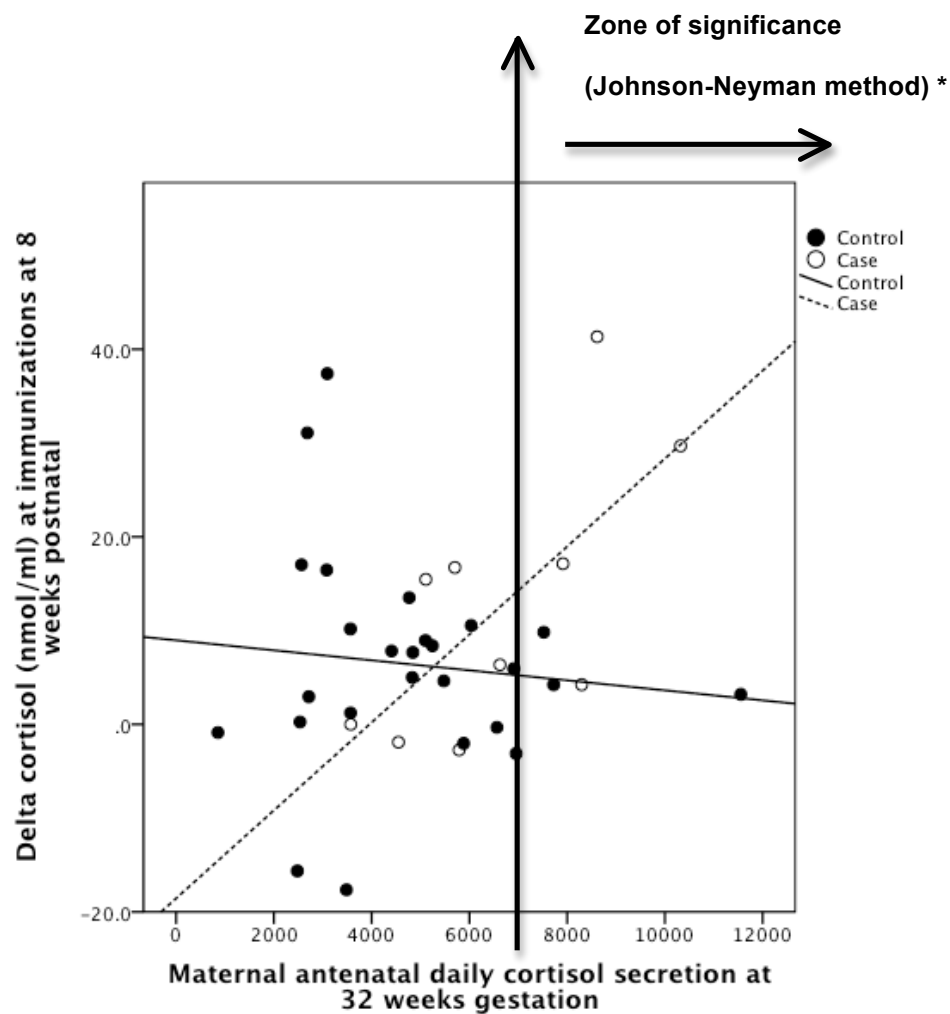


Figure 24: Moderation of the effect of caseness on delta cortisol

Note. * When maternal antenatal diurnal cortisol, at 32 weeks gestation, was ≥ 6938.6 nmol/ml², delta cortisol was statistically significantly higher in infants exposed to depression *in utero* compared with infants not so exposed.

3.5.4 Infant basal cortisol levels at 8 weeks postnatal (day 2)

To test the hypotheses that compared with 8-week-old infants not exposed to depression *in utero*, 8-week-old infants so exposed would have higher basal (awakening and evening) cortisol levels, infants of cases and controls were compared. 61 infants provided saliva samples in the morning and the evening the day after routine immunizations at 8 weeks postnatal. The saliva sampling details and cortisol are presented followed by the characteristics of infants and their mothers.

3.5.4.1 Saliva sampling details

Awakening and evening cortisol levels at 8 weeks postnatal were measured and saliva sampling details were recorded and their associations examined. There was no significant correlation between infant awakening cortisol and any saliva sampling detail (awakening and sample time, interval between awakening and sample, interval between the end of the last feed and sample time, and the interval between sample acquisition and freezing) (see Appendix P, Table 78). Nor was there a significant correlation between infant evening cortisol and saliva sampling details (sample time, interval between the end of the last feed and sample time, interval between the end of the last nap and sample time, duration of the last nap before the evening sample and the interval between sample acquisition and freezing) (see Appendix P, Table 79).

3.5.4.2 Infant basal cortisol levels at 8 weeks postnatal and maternal antenatal depression

Compared with infants not exposed to depression in utero, infants who were so exposed had higher awakening cortisol; however, the difference (-4.11 nmol/ml, 95% CI [-4.1, 3.3]) was not statistically significant and represented only a small sized effect, $r = .13$. There was no statistically significant difference between the two groups in evening cortisol and a negligible sized effect, $r = .06$ (Table 35).

Table 35: Infant awakening and evening cortisol at 8 weeks postnatal and maternal antenatal depression

	Control	Case	Statistical test and
	(n = 37)	(n = 24)	significance
Infant awakening cortisol (nmol/ml), M (SD)			
	9.80 (8.67)	13.91 (17.33)	$t_{(59)} = 1.0, p = .30$
Infant evening cortisol (nmol/ml), M (SD)			
	8.35 (14.52)	7.47 (16.75)	$z = -0.5, p = .59$

3.5.5 Associations of infant basal cortisol levels at 8 weeks postnatal with maternal antenatal HPA axis

To test the hypotheses that infant HPA axis function at 8 weeks postnatal would be associated with maternal antenatal HPA axis, correlations were performed to examine associations between infant awakening and evening cortisol at 8 weeks postnatal and the maternal HPA axis measures that differed between cases and controls in pregnancy (CRH, controlling for gestational age at sample acquisition and awakening, evening and diurnal cortisol and CAR (AUCi) at 32 weeks gestation); there were no significant associations (Table 36).

Despite the absence of any significant correlation between these maternal antenatal HPA axis measures and infant basal cortisol, it is theoretically possible that mediation was taking place, thus I used PROCESS to examine this phenomenon further. However, I found no support for the hypothesis that measures of the maternal antenatal HPA axis were mediating an association of caseness with infant basal cortisol at 8 weeks postnatal.

Furthermore, using PROCESS, I found no evidence that the association between caseness and infant basal cortisol levels at 8 weeks postnatal was moderated by any of the antenatal HPA axis measures (CRH or awakening, evening or diurnal cortisol or CAR (AUCi) at 32 weeks gestation).

3.5.5.1 Summary of Associations of infant basal cortisol levels at 8 weeks postnatal with maternal antenatal HPA axis

There were no significant correlations between basal cortisol measures in 8-week-old infants and the maternal antenatal HPA axis measures that differed between cases and controls. Furthermore there was no evidence of mediation or moderation of the effect of caseness on these infant measures.

Table 36: Infant basal cortisol at 8 weeks postnatal and maternal antenatal HPA axis

Maternal antenatal HPA axis					
	CRH controlling for gestational age at sample acquisition	Awakening cortisol at 32 weeks gestation	Evening cortisol at 32 weeks gestation	Diurnal cortisol at 32 weeks gestation	CAR (AUCi) at 32 weeks gestation
Awakening	-.17	-.04	-.15	-.07	-.29
Evening	-.10	-.16	-.22	-.29	.11
n	54	49	48	43	22

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis.

Awakening = infant awakening cortisol

Evening = infant evening cortisol

3.6 Infant HPA axis at 1 year postnatal

3.6.1 Study subjects

At one year postnatal a further nine subjects had dropped out of the study leaving 66 (80%) subjects, 40 (87%) controls and 26 (72%) cases.

3.6.2 Infant cortisol response to the pain of immunization at 1 year postnatal (day 1)

To test the hypotheses that compared with 1-year-old infants not exposed, 1-year-old infants exposed to depression *in utero* would have a larger cortisol response to the pain of immunization; infants of cases and controls were compared. 43 infants provided saliva samples both before and 20 minutes after routine immunization at 1 year postnatal. The saliva sampling details and cortisol are presented followed by the characteristics of infants and their mothers.

3.6.2.1 Saliva sampling details

Cortisol levels before and 20 minutes after routine immunization at 1 year postnatal were measured. Saliva sampling details for this sample (time of sample before the immunization, time of the immunization, interval between the first sample and the immunization) were recorded. There were no significant correlations between the infant cortisol measures and any saliva sampling details at 1 year postnatal (see Appendix Q, Table 80).

3.6.2.2 Infant cortisol response to immunization at 1 year postnatal and maternal antenatal depression

Infants exposed to depression *in utero* were compared with those not so exposed. Non-parametric tests were used since there were outliers in both groups and the data were not normally distributed and did not benefit from transformation; the standard deviations were large in both groups demonstrating a large degree of variation in these infant cortisol measures. Cortisol after the immunization was higher in infants exposed to depression *in utero* compared with those not so exposed; this difference was statistically significant and it represented a medium sized effect, $r = .32$ (Figure 25Error! Reference source not found.). Delta cortisol was also higher in infants exposed to depression *in utero* compared with those not so exposed; this difference was not statistically significant, however it represented a small to medium sized effect, $r = 0.22$. Cortisol before the immunization was lower in infants exposed to depression *in utero* compared

with those not so exposed, however, this difference was not statistically significant and represented only a small sized effect, $r = .09$.

As at 8 weeks postnatal, on visual inspection the change in cortisol from before to after the immunization appeared different between infants of cases and controls (Figure 26), hence further examination of the data was warranted and the Wilcoxon signed-rank test was used. For the cases group there was a statistical trend for higher cortisol levels after than before the immunization (median, 5.34 v 3.63 respectively, $z = -1.71$, $p = .088$); this represented a medium sized effect, $r = .30$. For the control group the cortisol levels were not significantly different (median = 3.93 after v 3.16 before, $z = -0.32$, $p = .75$); this represented a negligible effect, $r = .04$ (Table 37). Thus there was a statistical trend for a medium sized effect of immunization on cortisol in infants exposed to depression *in utero* and no meaningful effect in infants not so exposed.

Table 37: Infant cortisol response to immunization at 1 year postnatal and maternal antenatal depression

	Control	Case	Statistical test
	(n = 27)	(n = 16)	and significance
Cortisol pre-immunization (nmol/ml), M (SD)			
	6.74 (15.20)	5.65 (4.78)	$z = -0.6, p = .55$
Cortisol post-immunization (nmol/ml), M (SD)			
	7.00 (15.21)	11.32 (19.82)	$z = -2.1, p = .038$
Delta cortisol (nmol/ml), M (SD)			
	0.27 (2.75)	5.67 (16.84)	$z = -1.4, p = .14$
Effect size for the change in cortisol from pre- to post-immunization			
	No effect	Medium sized effect	
	$r = .04, p = .75$	$r = .30, p = .088$	

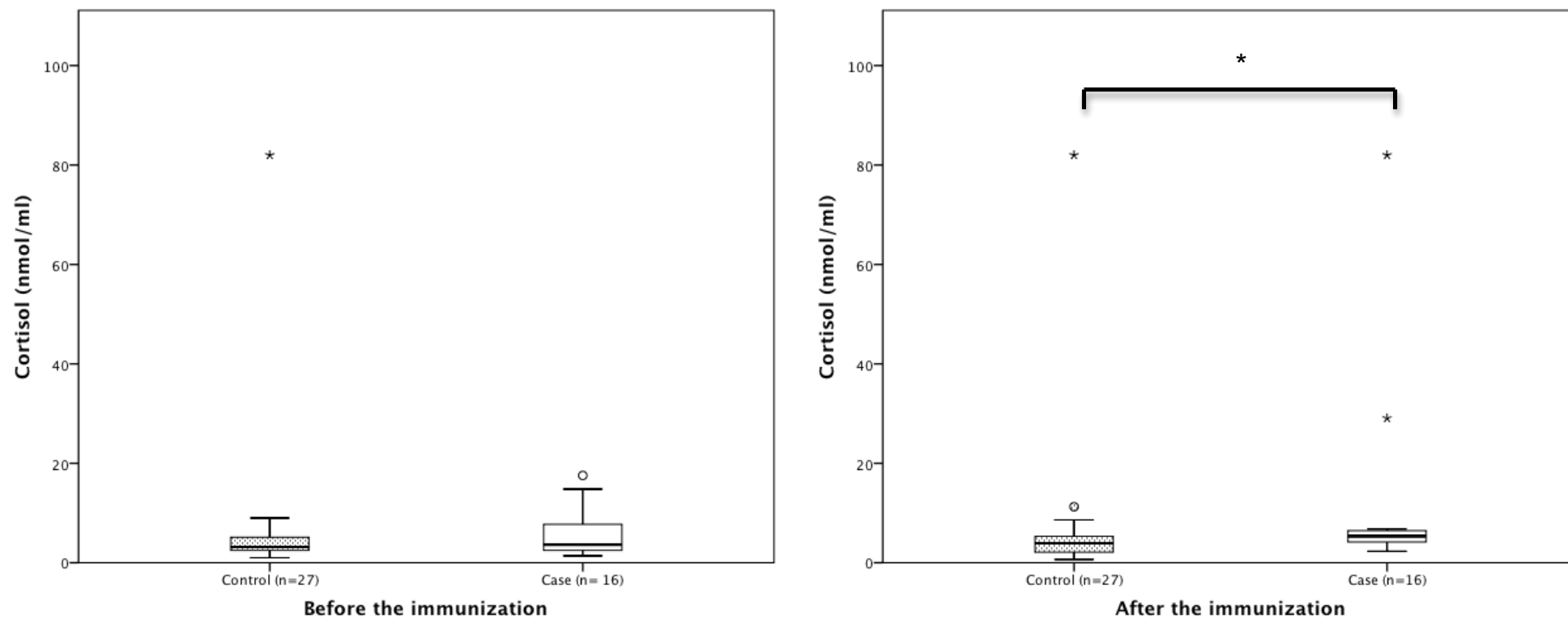


Figure 25: Infant cortisol before and 20 minutes after the immunization at 1 year postnatal

Note. *p=.038

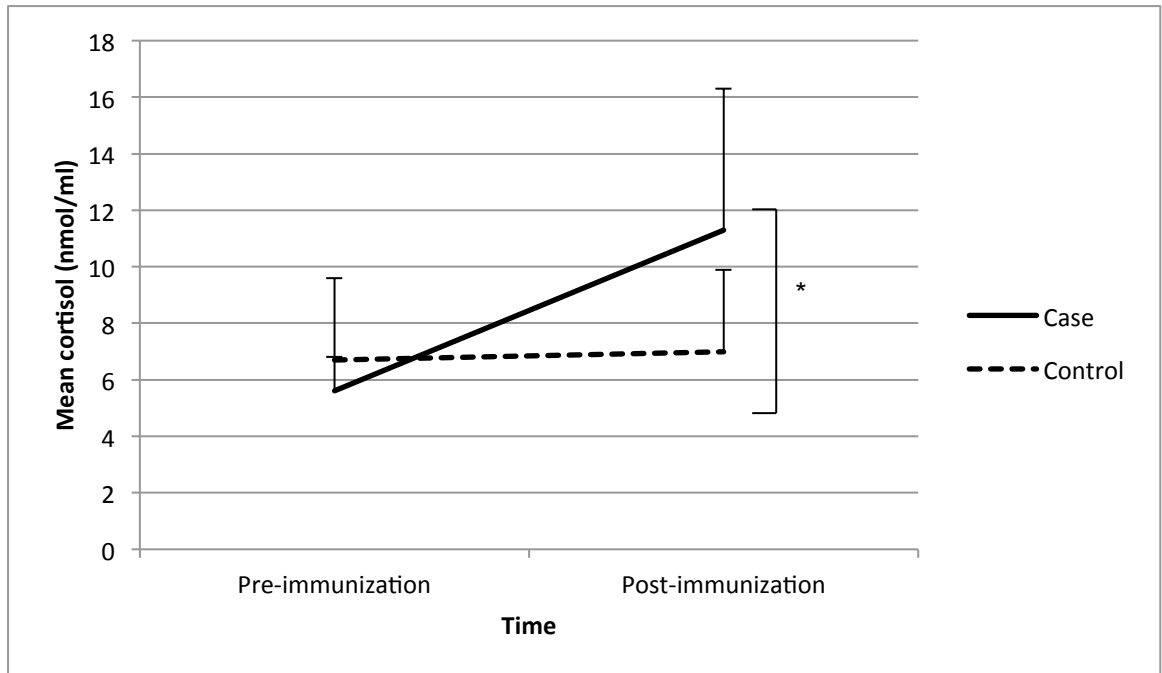


Figure 26: Infant cortisol response to immunization at 1 year postnatal

Note. *Cases, medium effect size ($p = .088$)

3.6.2.3 Infant cortisol response to immunization at 1 year postnatal and other infant and maternal characteristics

The characteristics of infants who provided saliva samples on the day of immunization at 1 year postnatal are presented; maternal factors are also presented since they are potential confounding factors in the analysis of infant cortisol. Infants of cases (thus exposed to antenatal depression *in utero*) and infants of controls (not exposed to antenatal depression *in utero*) were compared.

3.6.2.3.1 Infant characteristics at 1 year postnatal

There were no significant group differences in infant age at assessment, breastfeeding, health or medication use, or in Bayley scores for cognitive, language or motor development at 1 year postnatal (Table 38).

3.6.2.3.2 Infant factors prior to 1 year postnatal

Compared with infants of controls, infants of cases had been exposed to higher levels of symptoms of anxiety *in utero* (STAIS, M (SD) 28.6 (6.6) v 48.9 (14.0) respectively), this difference was statistically significant, $t_{(16.1)} = 5.1$, $p < .001$ and represented a large sized effect, $r = .78$. However, there was no significant group difference in exposure to antidepressants or smoking *in utero*, mode of delivery, gestational age at birth or the sex of the baby (Table 39).

3.6.2.3.3 Maternal characteristics (Table 40)

3.6.2.3.3.1 Maternal medication and health behaviours

Compared with controls, a larger proportion of cases were taking regular medication at 1 year postnatal (% (n), 8 (2) v 37.5 (6) respectively, $\chi^2_{(1)} = 5.4$, $p = .04$, OR = 6.9, 95% CI [1.2, 40.3]). There were no significant group differences in maternal health behaviours at 1 year postnatal.

3.6.2.3.3.2 Maternal mental health

There was no significant difference between cases and controls in a current diagnosis of MDD. Compared with controls the duration of MDD between the 8 week postnatal and 1 year postnatal assessments was significantly higher in cases (months M (SD), 0.03 (0.15) v 1.78 (3.07) respectively), this difference was statistically significant, $z = -3.0$, $p = .002$ and represented a medium to large sized effect, $r = .47$.

3.6.2.3.3 Maternal cortisol

Compared to controls, maternal awakening cortisol at 1 year postnatal was lower in cases (nmol/ml, M (SD), 10.48 (5.12) v 6.96 (3.0) respectively), this difference was statistically significant, $t_{(36)} = 2.4$, $p = .022$ and represented a medium sized effect, $r = .37$. There was no significant correlation between maternal awakening cortisol level and any saliva sampling details at 1 year postnatal, nor with any maternal factors which differed between cases and controls. There were no other group differences in any other maternal cortisol measure (evening, diurnal, AUCg and AUCi) at 1 year postnatal.

3.6.2.3.4 Summary of infant and maternal characteristics

As described above there were significant differences between cases and controls in a number of infant and maternal factors (exposure to symptoms of anxiety *in utero*, maternal duration of postnatal depression, concurrent use of medication and maternal awakening cortisol). However, further analyses showed that none of these differences was associated with the infant cortisol measures at immunization at 1 year postnatal; thus they were not accounting for the differences found in infant cortisol response to immunization (see Appendix Q, Table 81).

3.6.2.4 Summary of infant cortisol response to immunizations and antenatal depression at 1 year postnatal

In this sample of 43 infants with cortisol data before and 20 minutes after routine immunizations at 1 year postnatal, I found that compared with infants not exposed to depression *in utero*, infants who were exposed to depression *in utero* had a higher cortisol level after the immunizations, the difference was statistically significant and represented a medium sized effect. Furthermore, delta cortisol was also higher in infants exposed to depression *in utero*, this difference was not statistically significant, however it represented a small to medium sized effect. Moreover, immunization was associated with a trend for increase in cortisol equivalent to a medium effect size in cases but immunization had no effect on cortisol in controls. These findings were independent of other infant or maternal factors.

Table 38: Infant characteristics at 1 year postnatal (day 1)

	Control	Case	Statistical test and
	(n = 25 - 27)	(n = 16)	significance
Baby's age at assessment (months), M (SD)			
	12.7 (0.6)	12.8 (1.6)	t ₍₄₀₎ = 0.4, p = .75
Receiving any breast milk at 1 year postnatal, % (n)			
Yes	24.0 (6)	31.3 (5)	χ ² ₍₁₎ = 0.3, p = .72 ¹
No	76.0 (19)	68.8 (11)	
Any chronic illness at 1 year postnatal, % (n) ^{2, 3}			
Yes	8.3 (1)	16.7 (2)	χ ² ₍₁₎ = 0.4, p = 1.0 ⁴
No	91.7 (11)	83.3 (10)	
Taking regular medication at 1 year postnatal, % (n) ⁵			
Yes	16.0 (4)	6.3 (1)	χ ² ₍₁₎ = 0.9, p = .63 ⁴
No	84.0 (21)	93.8 (15)	
Taking PRN medication at 1 year postnatal, % (n) ⁶			
	0	0	
Bayley cognitive composite score, M (SD)			
	112.5 (16.1)	111.2 (12.8)	t ₍₄₀₎ = 0.2, p = .79
Bayley language composite score, M (SD)			
	104.5 (13.0)	97.7 (12.7)	t ₍₄₀₎ = 1.6, p = .11
Bayley motor composite score, M (SD)			
	102.6 (12.0)	102.7 (13.7)	t ₍₄₀₎ = 0.0, p = .98

¹ Fisher's exact test was applied as 1 cell had an expected cell count less than 5.² These data were not ascertained on the full sample (controls n=12, cases n= 12).³ Asthma, eczema, lactose intolerance⁴ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.⁵ Ventolin, cream for eczema, canestan cream, laxative.⁶ These data were not ascertained on the full sample (controls n=11, cases n= 11).

Table 39: Infant factors prior to 1 year postnatal (day 1)

	Control	Case	Statistical test and significance
	(n = 27)	(n = 16)	
Exposed to antidepressants <i>in utero</i>, % (n)¹			
No	100 (27)	84.6 (11)	$\chi^2_{(1)} = 4.4, p = .10^2$
Yes	0	15.4 (2)	
Exposed to smoking <i>in utero</i>, % (n)			
No	96.3 (26)	75.0 (12)	$\chi^2_{(1)} = 4.4, p = .06^2$
Yes	3.7 (1)	25.0 (4)	
Exposure to symptoms of anxiety <i>in utero</i> (STAIS score), M (SD)			
	28.6 (6.6)	48.9 (14.0)	$t_{(16.1)} = 5.1, p < .001$
Vaginal mode of delivery, % (n)			
Yes	66.7 (18)	81.3 (13)	$\chi^2_{(1)} = 1.1, p = .48$
No	33.3 (9)	18.8 (3)	
Gestational age at birth (weeks), M (SD)			
	40.32 (1.71)	39.90 (1.16)	$t_{(41)} = 0.8, p = .40$
Sex of the baby, % (n)			
Male	55.6 (15)	68.8 (11)	$\chi^2_{(1)} = 0.7, p = .39$
Female	44.4 (12)	31.3 (5)	

¹ These data were not ascertained on the full sample of cases (n= 13).

² Fisher's exact test was applied as 2 cells had an expected cell count less than 5

Table 40: Maternal characteristics at 1 year postnatal (day 1)

	Control (n = 24 - 27)	Case (n = 14 - 16)	Statistical test and significance
Taking regular medication at 1 year postnatal, % (n)¹			
Yes	8.0 (2)	37.5 (6)	$\chi^2_{(1)} = 5.4, p = .04^2$
No	92.0 (23)	62.5 (10)	
Taking regular or PRN steroid medication) at 1 year postnatal, % (n)³			
Yes	8.3 (1)	16.7 (2)	$\chi^2_{(1)} = 0.4, p = 1.0^2$
No	91.7 (11)	83.3 (10)	
Coffee (cups/week) at 1 year postnatal, mean (SD)⁴			
	6.9 (6.9)	5.3 (7.9)	$z = -0.9, p = .39$
Tea (cups/week) at 1 year postnatal, mean (SD)⁴			
	10.6 (11.4)	6.2 (6.2)	$t_{(15.2)} = 1.1, p = .26$
Alcohol use (units/week) at 1 year postnatal, % (n)⁵			
	4.0 (4.8)	0.8 (1.0)	$z = -1.7, p = .10$
Cigarette smoking at 1 year postnatal, % (n)⁴			
Yes	9.1 (1)	25.0 (3)	$\chi^2_{(1)} = 1.0, p = .59^2$
No	90.9 (10)	75.0 (3)	
Current diagnosis of DSM-IV MDD, % (n)			
Yes	0	12.5 (2)	$\chi^2_{(1)} = 3.4, p = .14^2$
No	100 (27)	87.5 (14)	
Duration of MDD between 2 and 12 months postnatal (months), M (SD)			
	0.03 (0.15)	1.78 (3.07)	$z = -3.0, p = .002$
Awakening cortisol at 1 year postnatal (nmol/ml), M (SD)⁶			
	10.48 (5.12)	6.96 (3.00)	$t_{(36)} = 2.4, p = .022$
Evening cortisol at 1 year postnatal (nmol/ml), M (SD)⁷			
	1.72 (3.36)	1.29 (0.83)	$z = -0.9, p = .37$

¹ Thyroxine, antibiotic eye drops, antihistamine, OCP, steroid inhaler, sertraline, imipramine, fluoxetine, ranitidine, loratidine, non-steroid asthma inhaler

² Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

³ Not ascertained on full sample (controls n=12, cases n=12)

⁴ Not ascertained on full sample (controls n=11, cases n=12)

⁵ Not ascertained on full sample (controls n=11, cases n=8)

⁶ Awakening cortisol was not ascertained on the whole sample (controls n = 23 cases n =15)

⁷ Evening cortisol was not ascertained on the whole sample (controls n = 22 cases n =12)

Diurnal cortisol at 1 year postnatal (nmol/ml ²), M (SD) ¹		
3891.0 (1589.6)	3450.8 (1314.5)	t ₍₂₈₎ = 0.8, p = .43
Post-awakening cortisol (AUCg) at 1 year postnatal (nmol/ml ²), M (SD) ²		
602.3 (218.6)	624.3 (230.5)	t ₍₁₀₎ = 0.2, p = .87
CAR (AUCi) at 1 year postnatal (nmol/ml ²), M (SD) ²		
83.9 (201.8)	162.8 (142.4)	t ₍₁₀₎ = 0.8, p = .45

¹ Diurnal cortisol was not ascertained on the whole sample (controls n = 18 cases n = 12)

² CAR was not ascertained on the whole sample (controls n = 6 cases n = 6)

3.6.3 Associations of infant cortisol response to immunizations at 1 year postnatal with maternal antenatal HPA axis

To test the hypotheses that infant HPA axis function at 1 year postnatal would be associated with maternal antenatal HPA axis, correlations were performed to examine associations between infant cortisol (before and after immunizations and the change in cortisol) at 1 year postnatal and the maternal antenatal HPA axis measures that differed between cases and controls (CRH, controlling for gestational age at sample acquisition and awakening, evening and diurnal cortisol and CAR (AUCi) at 32 weeks gestation) (see Table 41).

Infant cortisol before the immunization was significantly negatively correlated with maternal awakening cortisol at 32 weeks gestation ($r_s = -.38$, $p = .028$) and with diurnal cortisol secretion (AUC) at 32 weeks gestation ($r_s = -.49$, $p = .006$). These findings demonstrate a degree of continuity, since, as described in section 3.2.2 above, compared with controls, cases had significantly higher awakening and diurnal cortisol (AUC) at 32 weeks gestation and infants of cases had lower cortisol before the immunizations than infants of controls, although this was not statistically significant.

Infant cortisol 20 minutes after the immunization was positively correlated with maternal evening cortisol at 32 weeks gestation at a trend level of significance ($r_s = .33$, $p = .060$). Again, these findings demonstrate a degree of continuity, since, as described in section 3.2.2 above, compared with controls, cases had statistically significant higher evening cortisol at 32 weeks gestation and infants of cases had statistically significant higher cortisol after the immunizations than infants of controls.

The change (delta) in infant cortisol from before to 20 minutes after the immunization was significantly positively correlated with maternal evening cortisol at 32 weeks gestation ($r_s = .48$, $p = .004$) and with diurnal cortisol secretion (AUC) at 32 weeks gestation ($r_s = .47$, $p = .009$). Again, these findings demonstrate a degree of continuity, since, as described in section 3.2.2 above, compared with controls, cases had significantly higher evening and diurnal cortisol (AUC) at 32 weeks gestation and infants of cases had a greater delta cortisol than infants of controls, although this was not statistically significant.

There were no other significant correlations between the infant and maternal cortisol measures (Table 41).

PROCESS was used to test for mediation, which showed that maternal antenatal cortisol measures had no indirect effect on the association between caseness and infant cortisol response to immunizations at 1 year postnatal. Furthermore, there were no significant interactions between caseness and maternal antenatal cortisol, indicating that maternal antenatal cortisol was not moderating this association at 1 year postnatal.

3.6.3.1 Summary of associations of infant cortisol response to immunizations at 1 year postnatal with maternal antenatal HPA axis

There were a number of significant correlations between cortisol response to immunization in 1-year-old infants and the maternal antenatal HPA axis measures that differed between cases and controls, as described in section 3.6.3 above. These correlations demonstrate continuity between maternal antenatal cortisol and infant cortisol at 1 year of age since the direction of the correlation corresponded with the direction of the group difference in maternal antenatal cortisol in relation to that found in the infant cortisol. However, there was no evidence of mediation or moderation of the effect of caseness on these infant measures.

Table 41: Infant cortisol response to immunizations at 1 year postnatal and maternal antenatal HPA axis

Maternal antenatal HPA axis					
	CRH controlling for gestational age at sample acquisition	Awakening cortisol at 32 weeks gestation	Evening cortisol at 32 weeks gestation	Diurnal cortisol at 32 weeks gestation	CAR (AUCi) at 32 weeks gestation
Pre	-.04	-.38*	-.17	-.49**	-.32
Post	.01	-.07	.33[∞]	.05	-.19
Delta	.05	.28	.48**	.47**	.25
n	37	33	34	30	16

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis.

[∞] p = .060, *p <.05, **p <.01

Pre = cortisol before the immunizations

Post = cortisol 20 minutes after the immunizations

Delta = change in cortisol from before to after the immunizations

3.6.4 Infant basal cortisol levels at 1 year postnatal (day 2)

To test the hypotheses that compared with 1-year-old infants not exposed to depression *in utero*, 1-year-old infants so exposed would have higher basal (awakening and evening) cortisol levels, infants of cases and controls were compared. 53 infants provided saliva samples in the morning and the evening the day after routine immunizations at 1 year postnatal. The saliva sampling details and cortisol are presented followed by the characteristics of infants and their mothers.

3.6.4.1 Saliva sampling details

Awakening and evening cortisol levels at 1 year postnatal were measured and the saliva sampling details were recorded. There was no significant correlation between infant awakening cortisol and any saliva sampling detail (awakening and sample time, interval between awakening and sample, interval between the end of the last feed and sample time, and the interval between sample acquisition and freezing (see Appendix Q, Table 82). Nor was there a significant correlation between infant evening cortisol and saliva sampling details (sample time, interval between the end of the last feed and sample time, interval between the end of the last nap and sample time, duration of the last nap before the evening sample and the interval between sample acquisition and freezing) (see Appendix Q, Table 83).

3.6.4.2 Infant basal cortisol levels at 1 year postnatal and maternal antenatal depression

Infants exposed to depression *in utero* were compared with those not so exposed. Non-parametric tests were used since there were outliers in both groups and the data were not normally distributed and did not benefit from transformation; the standard deviations were large in both groups demonstrating a large degree of variation in these infant cortisol measures. Cortisol after the immunization was higher in infants exposed to depression *in utero* compared with those not so exposed. Compared with 1-year-old infants not exposed to depression *in utero*, 1-year-old infants who were exposed had higher evening cortisol (M (SD), 4.66 nmol/ml (12.22) v 12.66 nmol/ml (24.80)); this difference was statistically significant and represented a medium sized effect, $r = .30$. However, compared with 1-year-old infants not exposed to depression *in utero*, although 1-year-old infants who were exposed had higher awakening cortisol (M (SD), 10.68

nmol/ml (13.69) v 11.66 nmol/ml (18.20)), this difference was not statistically significant and represented only a small sized effect, $r = .10$ (Table 42 and Figure 27).

Table 42: Infant basal cortisol at 1 year postnatal and maternal antenatal depression

	Control	Case	Statistical test and
	(n = 34)	(n = 19)	significance
Infant awakening cortisol (nmol/ml), M (SD)			
	10.68 (13.69)	11.66 (18.20)	$z = -0.7, p = .50$
Infant evening cortisol (nmol/ml), M (SD)			
	4.66 (12.22)	12.94 (24.80)	$z = -2.2, p = .029$

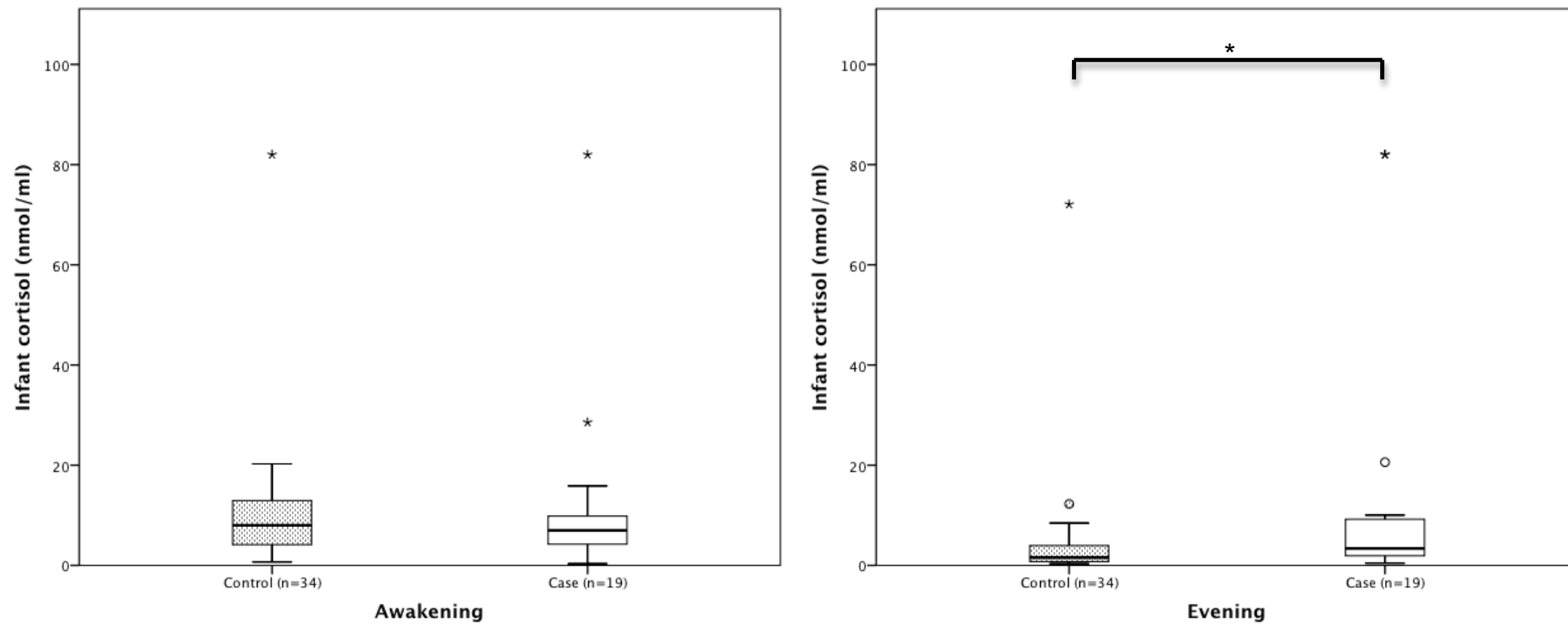


Figure 27: Infant basal cortisol at 1 year postnatal

Note. * $p = .029$

3.6.4.3 Infant basal cortisol levels at 1 year postnatal and other infant and maternal characteristics

The characteristics of infants who provided awakening and evening saliva samples the day after immunization at 1 year postnatal are presented; maternal factors are also presented, as they are potential confounding factors in the analysis of infant cortisol. Infants of cases (thus exposed to antenatal depression *in utero*) and infants of controls (not exposed to antenatal depression *in utero*) were compared.

3.6.4.3.1 Infant characteristics at 1 year postnatal

There was no significant group difference in infant age at assessment, breastfeeding, health or medication use, or in Bayley scores for cognitive, language or motor development at 1 year postnatal (Table 43).

3.6.4.3.2 Infant factors prior to 1 year postnatal

Compared with infants of controls, infants of cases had been exposed to higher levels of symptoms of anxiety *in utero* (STAIS, M (SD), 26.5 (6.8) v 49.6 (11.6) respectively), this difference was statistically significant, $z = -5.2$, $p < .001$ and represented a large sized effect, $r = .74$. There was no significant group difference in exposure to antidepressants *in utero*, smoking *in utero*, mode of delivery, gestational age at birth or the sex of the infant (Table 44).

3.6.4.3.3 Maternal characteristics (Table 45)

3.6.4.3.3.1 Maternal medication and health behaviours

There were no significant group differences in any maternal medication or health behaviours at 1 year postnatal.

3.6.4.3.3.2 Maternal mental health

Compared with controls, a greater proportion of cases had a current diagnosis of MDD at 1 year postnatal (% (n), 0 (34) v 15.8 (3), this difference was statistically significant, $\chi^2_{(1)} = 5.7$, $p = .041$. Furthermore, compared with controls the duration of MDD between the 8 week postnatal and 1 year postnatal assessments was significantly higher in cases (months M (SD), 0.02 (0.13) v 2.11

(3.61) respectively), this difference was statistically significant, $z = -3.3$, $p = .001$ and represented a medium to large sized effect, $r = .47$.

3.6.4.3.3 Maternal cortisol

There was no significant difference, between cases and controls, in any maternal cortisol measure at 1 year postnatal.

3.6.4.3.4 Summary of infant and maternal characteristics

As described above there were significant differences between cases and controls in infant and maternal factors (exposure to symptoms of anxiety *in utero*, current diagnosis of MDD and duration of postnatal depression). Further analyses showed that neither exposure to symptoms of anxiety *in utero* nor the duration of postnatal depression were associated with the infant basal cortisol measures at 1 year postnatal. The small number of subjects ($n=3$) with MDD at 1 year postnatal precluded further analysis. Nonetheless, in order to examine this association further, depression symptom scores (according to BDI score at 1 year postnatal) were examined. There was no association between infant awakening cortisol and BDI score (either for all subjects, or for cases and controls separately, $r_s = .04$, $p = .80$, $r_s = .00$, $p = .99$ and $r_s = .12$, $p = .50$ respectively). Furthermore, there was no association between infant evening cortisol and BDI score (either for all subjects, or for cases and controls separately, $r_s = .23$, $p = .10$, $r_s = .27$, $p = .27$ and $r_s = .07$, $p = .68$ respectively); thus these differences did not seem to be accounting for the differences found in infant basal cortisol, see Appendix Q, Table 84. There were no other group differences, in infant or maternal factors, between cases and controls.

3.6.4.4 Summary of infant basal cortisol levels and antenatal depression at 1 year postnatal

In this sample of 53 infants with cortisol data at awakening and evening at 1 year postnatal, I found that evening cortisol was significantly higher in infants of cases (thus exposed to depression *in utero*), than in infants of controls; these findings appeared to be independent of other maternal and infant factors (Figure 25Error! Reference source not found.).

Table 43: Infant characteristics at 1 year postnatal (day 2)

	Control	Case	Statistical test and significance
	(n = 33 - 34)	(n = 18 - 19)	
Baby's age at assessment (months), M (SD)			
	12.7 (0.6)	12.7 (0.8)	$z = -0.5, p = .64$
Receiving any breast milk at 1 year postnatal, % (n)			
Yes	24.2 (8)	33.3 (6)	$\chi^2_{(1)} = 0.5, p = .52^1$
No	75.8 (25)	66.7 (12)	
Any chronic illness, % (n)^{2, 3}			
Yes	5.9 (1)	8.3 (1)	$\chi^2_{(1)} = 0.1, p = 1.0^4$
No	94.1 (16)	91.7 (11)	
Taking regular medication, % (n)⁵			
Yes	18.2 (6)	5.6 (1)	$\chi^2_{(1)} = 1.6, p = .40^4$
No	81.8 (27)	94.4 (17)	
Bayley cognitive composite score, M (SD)			
	113.24 (14.6)	108.4 (12.2)	$t_{(51)} = 1.2, p = .23$
Bayley language composite score, M (SD)			
	102.3 (10.7)	99.2 (12.0)	$t_{(51)} = 1.0, p = .33$
Bayley motor composite score, M (SD)			
	100.9 (11.3)	100.3 (12.2)	$t_{(51)} = 0.2, p = .86$

¹ Fisher's exact test was applied as 1 cell had an expected cell count less than 5.

² These data were not ascertained on the full sample (controls n=17, cases n= 12).

³ Asthma, lactose intolerance

⁴ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

⁵ Atrovent, ventolin, cream for eczema, canestan cream, laxative.

Table 44: Infant factors prior to 1 year postnatal (day 2)

	Control (n = 33 - 34)	Case (n = 18 - 19)	Statistical test and significance
Exposed to antidepressants <i>in utero</i>, % (n)¹			
Yes	0	13.3 (2)	$\chi^2_{(1)} = 4.7$, p = .09 ²
No	100 (34)	86.7 (13)	
Exposed to smoking <i>in utero</i>, % (n)			
Yes	6.1 (2)	27.8 (5)	$\chi^2_{(1)} = 4.6$, p = .08 ²
No	93.9 (31)	72.2 (13)	
Exposure to symptoms of anxiety <i>in utero</i> (STAIS score), M (SD)			
	26.5 (6.8)	49.6 (11.6)	z = 5.2, p < .001
Vaginal mode of delivery, % (n)			
Yes	70.6 (24)	73.7 (14)	$\chi^2_{(1)} = 0.1$, p = .81
No	29.4 (10)	26.3 (5)	
Gestational age at birth (weeks), M (SD)			
	40.36 (1.55)	40.19 (1.16)	z = -0.7, p = .48
Sex of the baby, % (n)			
Male	52.9 (18)	52.6 (10)	$\chi^2_{(1)} = 0.0$, p = .98
Female	47.1 (16)	47.4 (9)	

¹ Not ascertained on full sample of cases (n=15)² Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

Table 45: Maternal characteristics at 1 year postnatal (day 2)

	Control (n = 32 - 34)	Case (n = 18 - 19)	Statistical test and significance
Taking regular medication at 1 year postnatal, % (n)¹			
Yes	12.1 (4)	27.8 (5)	$\chi^2_{(1)} = 2.0, p = .25^2$
No	87.9 (29)	72.2 (13)	
Taking regular or PRN steroid medication, % (n)³			
Yes	25.0 (5)	16.7 (2)	$\chi^2_{(1)} = 0.3, p = .68^4$
No	75.0 (15)	83.3 (10)	
Coffee (cups/week) at 1 year postnatal, mean (SD)⁵			
	7.1 (7.0)	10.5 (13.2)	$z = -0.2, p = .87$
Tea (cups/week) at 1 year postnatal, mean (SD)⁵			
	12.5 (11.8)	9.0 (9.9)	$z = -0.7, p = .48$
Alcohol use (units/day) at 1 year postnatal, M (SD)⁶			
	4.4 (4.4)	0.9 (1.1)	$z = -2.0, p = .06$
Cigarette smoking at 1 year postnatal, % (n)⁵			
Yes	12.5 (2)	25.0 (3)	$\chi^2_{(1)} = 0.7, p = .62^4$
No	87.5 (14)	75.0 (3)	
Current diagnosis of DSM-IV MDD, % (n)			
Yes	0	15.8 (3)	$\chi^2_{(1)} = 5.7, p = .041^4$
No	100 (34)	84.2 (16)	
Duration of MDD between 2 and 12 months postnatal (months), M (SD)			
	0.02 (.13)	2.11 (3.61)	$z = -3.3, p = .001$
Awakening cortisol at 1 year postnatal (nmol/ml), M (SD)			
	10.02 (4.73)	7.32 (2.96)	$z = -1.9, p = .052$
Evening cortisol at 1 year postnatal (nmol/ml), M (SD)⁷			
	1.60 (2.90)	1.34 (0.77)	$z = -1.0, p = .30$

¹ Thyroxine, antibiotic eye drops, becotide, flixotide, serevent, ranitidine, thyroxine, antihistamine, OCP, sertraline, fluoxetine

² Fisher's exact test was applied as 1 cell had an expected cell count less than 5.

³ Not ascertained on full sample (controls n=20, cases n=12)

⁴ Fisher's exact test was applied as 2 cells had an expected cell count less than 5.

⁵ Not ascertained on full sample (controls n=16, cases n=12)

⁶ Not ascertained on full sample (controls n=15, cases n=7)

⁷ Evening cortisol was not ascertained on the whole sample (controls n = 30 cases n =14)

Diurnal cortisol at 1 year postnatal (nmol/ml ²), M (SD) ¹		
3794.5 (1550.2)	3580.1 (1015.9)	$t_{(37)} = 0.4, p = .66$
Post-awakening cortisol (AUCg) at 1 year postnatal (nmol/ml ²), M (SD) ²		
561.1 (245.4)	642.0 (185.8)	$t_{(17)} = 0.8, p = .43$
CAR (AUCi) at 1 year postnatal (nmol/ml ²), M (SD) ²		
49.7 (157.2)	160.6 (189.6)	$z = -1.8, p = .07$

¹ Diurnal cortisol was not ascertained on the whole sample (controls n = 26 cases n = 13)

² CAR was not ascertained on the whole sample (controls n = 10 cases n = 9)

3.6.5 Associations of infant basal cortisol levels at 1 year postnatal with maternal antenatal HPA axis

To test the hypotheses that infant HPA axis function at 1 year postnatal would be associated with maternal antenatal HPA axis, correlations were performed to examine associations between infant awakening and evening cortisol levels at 1 year postnatal and the maternal HPA axis measures that differed between cases and controls in pregnancy (CRH, controlling for gestational age at sample acquisition and awakening, evening and diurnal cortisol and CAR (AUCi) at 32 weeks gestation); there were no significant associations (Table 46).

Despite the absence of any significant correlation between these maternal antenatal HPA axis measures and infant basal cortisol, it is theoretically possible that mediation was taking place, thus I used PROCESS to examine this phenomenon further. However, I found no support for the hypothesis that measures of the maternal antenatal HPA axis were mediating an association of caseness with infant basal cortisol at 1 year postnatal.

Furthermore, using PROCESS, I found no evidence that the association between caseness and infant basal cortisol levels at 1 year postnatal was moderated by any of the antenatal HPA axis measures (CRH, controlling for gestational age at sample acquisition or awakening, evening or diurnal cortisol or CAR (AUCi) at 32 weeks gestation).

3.6.5.1 Summary of associations of infant basal cortisol levels at 1 year postnatal with maternal antenatal HPA axis

There were no significant correlations between basal cortisol measures in 1-year-old infants and the maternal antenatal HPA axis measures that differed between cases and controls. Furthermore there was no evidence of mediation or moderation of the effect of caseness on these infant measures.

Table 46: Infant basal cortisol at 1 year postnatal and maternal antenatal HPA axis

Maternal antenatal HPA axis					
	CRH controlling for gestational age at sample acquisition	Awakening cortisol at 32 weeks gestation	Evening cortisol at 32 weeks gestation	Diurnal cortisol at 32 weeks gestation	CAR (AUCi) at 32 weeks gestation
Awakening	-.13	.18	.00	.16	.30
Evening	-.14	.21	.08	.18	.15
n	46	45	42	39	22

Note. Spearman's correlation coefficients are presented.

Awakening = infant awakening cortisol

Evening = infant evening cortisol

Chapter 4 DISCUSSION

4.1 Overview

A prospective longitudinal observational study design was used to examine the effects of antenatal depression on maternal HPA axis in pregnancy, obstetric outcomes and offspring HPA axis. A control group of 46 healthy pregnant women, and their offspring, were compared with a cases group of 36 women with a DSM-IV diagnosis of major depressive disorder in pregnancy, and their offspring, up to one year postnatal.

The cases group was defined by an operational diagnosis of depression rather than depression symptom scores because the study was intended to examine the effects of MDD in pregnancy. The effect of a disease, as opposed to symptoms of a disease, is more clinically meaningful, for example, in the ability to identify relevant cases and apply evidence-based treatment (O'Connor et al., 2013b). Furthermore, a focus on symptom scores, as opposed to diagnoses, may lead to type II error regarding any true effect of antenatal depression, since symptoms of depression have been shown to be more prevalent in pregnant women, compared to their matched, non-pregnant acquaintances, when concurrent assessment of operationally defined diagnoses of depression in the same women revealed the same prevalence in the pregnant and non-pregnant groups (O'Hara et al., 1990). Indeed, in support of this idea, the effect of antenatal depression on maternal cortisol was stronger for operationally defined diagnosis than for symptom severity when tested in the same study (O'Connor et al., 2013b).

Firstly, a summary of the main findings is presented, followed by a more detailed discussion of the individual findings, how they relate to the existing literature and a discussion of their implication. Finally the strengths and limitations are discussed along with suggestions for future research.

4.2 Summary of main findings

4.2.1 Antenatal depression and HPA axis in pregnancy

The findings demonstrate that, compared with women without antenatal depression, those with antenatal depression had an overactive HPA axis, including higher levels of CRH, awakening, evening and diurnal cortisol in the third trimester of pregnancy; they also had a blunted cortisol awakening response (AUCi). However, no group differences in HPA axis measures were apparent in the second trimester of pregnancy.

4.2.2 Antenatal depression and obstetric outcome

The findings demonstrate, in those with spontaneous onset of labour at term, that women with antenatal depression had a shorter length of gestation than women without antenatal depression. However, group differences were not apparent in other obstetric outcomes including birth weight, the proportion with PTB, LBW or SGA babies.

4.2.3 Antenatal depression, antenatal HPA axis and obstetric outcome

I found no evidence to support the hypothesis that the maternal antenatal HPA axis measures that differed between cases and controls were moderating or mediating the effect of caseness on gestational age at birth.

4.2.4 Antenatal depression and offspring HPA axis at 8 weeks postnatal

The findings demonstrate that there was a statistically significant increase in cortisol following immunization for both groups of infants. However the size of this effect was larger in infants exposed to depression *in utero* than in those not so exposed. There was no statistically significant group difference in cortisol after the immunizations, delta cortisol or basal cortisol measures.

4.2.5 Antenatal depression, antenatal HPA axis and offspring HPA axis at 8 weeks postnatal

The findings demonstrate that maternal antenatal cortisol moderated the effect of caseness on infant delta cortisol. Specifically, compared with infants who were not exposed to depression *in utero*, infants who were so exposed had higher delta cortisol when maternal antenatal diurnal cortisol was high. However, I found no correlations between maternal antenatal HPA axis measures and infant cortisol at 8 weeks postnatal nor did I find evidence that the maternal antenatal HPA axis measures that differed between cases and controls were moderating or mediating the effect of caseness on any other infant cortisol measure at 8 weeks postnatal.

4.2.6 Antenatal depression and offspring HPA axis at 1 year postnatal

The findings demonstrate that, compared with infants who were not exposed to depression *in utero*, infants who were so exposed had higher cortisol after the immunization. Furthermore, immunization was associated with a medium sized effect (increase) on cortisol in infants exposed to depression *in utero*. Conversely, immunization was not associated with an effect on cortisol in

infants who were not exposed to depression *in utero*. Lastly, compared with infants who were not exposed to depression *in utero*, infants who were so exposed had higher basal (evening) cortisol the day after immunization. However, the groups did not differ in delta cortisol or basal (awakening) cortisol.

4.2.7 Antenatal depression, antenatal HPA axis and offspring HPA axis at 1 year postnatal

The findings demonstrate that at 1 year postnatal there was a statistically significant negative correlation between cortisol before the immunization and maternal antenatal awakening and diurnal cortisol at 32 weeks gestation. Furthermore there was a statistically significant positive correlation between delta cortisol and maternal antenatal evening and diurnal cortisol and a statistical trend for positive correlation between cortisol after the immunization and maternal antenatal evening cortisol. However, I found no evidence that the maternal antenatal HPA axis measures that differed between cases and controls were moderating or mediating the effect of caseness on infant cortisol at 1 year postnatal.

4.2.8 Summary

In summary, the findings demonstrate that women with MDD in pregnancy had an overactive HPA axis in the third trimester (but not the second), a shorter length of gestation and offspring with altered HPA axis function at 8 weeks and 1 year postnatal. Furthermore, maternal antenatal diurnal cortisol moderated the infants' delta cortisol at 8 weeks postnatal, such that at higher levels of maternal diurnal cortisol infants exposed to MDD *in utero* had a greater delta cortisol than infants who were not so exposed. A number of significant correlations between maternal antenatal cortisol and infant cortisol measures at 1 year demonstrated a link between these two factors. However, there was no evidence that maternal antenatal HPA axis moderated or mediated the effect of caseness on infant cortisol measures at 1 year postnatal. Nor was there any evidence that the maternal antenatal HPA axis mediated the effect of caseness on gestational age at birth or on infant cortisol measures at 8 weeks. The findings were independent of other potential confounding factors.

4.3 Antenatal depression and HPA axis in pregnancy

I hypothesised that compared with healthy pregnant women those with antenatal depression would have overactivity of the HPA axis in the second and third trimesters of pregnancy. I found that compared with healthy women, the depressed group had higher levels of CRH, awakening, evening and diurnal cortisol but a blunted cortisol awakening response (AUCi) in the third trimester of pregnancy. However, group differences were not apparent in the second trimester of pregnancy.

In general, as described in section 1.4.5 above there is a paucity of literature on antenatal depression and maternal HPA axis in pregnancy. Furthermore, only a minority of these studies are of operationally defined MDD according to a diagnostic instrument. Some studies of operationally defined MDD had a larger sample size than the current study but they made only a single measure of cortisol either once or twice in pregnancy. Compared with other studies in this field, the study of O'Keane et al. has provided the broadest HPA axis assessment to date (O'Keane et al., 2011). In the following sections, I have considered each HPA axis measure in turn and described how it sits in the context of the existing literature; this is followed by a summary of the impact and implications of my findings.

4.3.1 Antenatal depression and CRH in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have higher CRH. My findings support this hypothesis, although this finding was only evident following moderation analyses, which showed that the effect of caseness on CRH was moderated by the gestational age at sample acquisition, such that the effect only became apparent when CRH was measured after 28.49 weeks gestation.

To my knowledge only one other study of antenatal depression and CRH has a cases group with MDD defined by diagnostic interview, and compared them with non-depressed pregnant women (O'Keane et al., 2011). In this study CRH was measured at both 25 and 36 weeks gestation, a significant group difference was found at 25 weeks ($n = 55$) and a statistical trend at 36 weeks ($n = 51$) gestation. However, no indication was provided of how gestational age at sample acquisition was determined or if there was any deviation from the stated week of sample acquisition; thus it is difficult to compare my findings. Nonetheless, on balance, my findings are

similar to those described, in that I found CRH was significantly higher in women with MDD compared with those without MDD. However, the gestational ages at which this finding was apparent differed between the two studies. I found a group difference only when CRH was measured after 28.74 weeks gestation; and (according to the Johnson-Neyman technique) the effect of caseness on CRH got larger as gestational age at sample acquisition increased, but no samples were taken as late as 36 weeks gestation. In summary my findings partially corroborate and further refine the existing literature since this thesis describes a more methodologically robust experiment with a larger sample size ($n=78$), clearly stated means of determining gestational age at sample acquisition and with precise documentation of this variable. Furthermore, it is the first replication, albeit partial, of the findings of O'Keane et al. As described in section 1.4.5.2 above the current study was conducted in the same research and clinical setting as O'Keane et al. but with an entirely separate sample of participants. Others have studied antenatal depression using self-rated depressive symptom scores at one time-point in pregnancy. One study of preterm birth showed no association between antenatal depression (defined by a CES-D score ≥ 16) and CRH (both measured at 24-26 weeks gestation) (Kramer et al., 2009). This study mirrors the findings described in this thesis, when no group difference was apparent at equivalent gestational ages. In contrast, another study showed that higher CRH (measured between 24.6 and 37.4 weeks gestation) was associated with higher odds of major or minor depression (defined by EPDS scores ≥ 13) (Rich-Edwards et al., 2008). Again, these findings appear to corroborate those described in this thesis.

4.3.2 Antenatal depression and CRHBP in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have lower CRHBP. My findings do not support this hypotheses; I found no significant group difference and only a small sized effect (cases > controls) for the group difference in CRHBP. The CRHBP protein is quite unstable, thus keeping the blood sample on ice and extracting plasma in a timely fashion and under cool conditions is critical and the protein is technically difficult to measure; this instability was perhaps reflected in the fact that CRHBP was at reliably detectable levels in only 38 subjects (50% of those who provided a blood sample). Thus the sample size was reduced considerably and the absence of a difference may be type II error; alternatively no difference exists. The interpretation of no difference is also reasonable since CRHBP is thought to be stable throughout normal pregnancy until a decline at about 36

weeks gestation and in this study CRHBP was measured earlier in gestation. However, CRHBP has been shown to be lower in the second and third trimesters of women who went on to have a preterm delivery compared with those who had full term births (Hobel et al., 1999). To my knowledge, there are no published studies of CRHBP in antenatal depression or even prenatal stress, but my hypothesis evolved from the established associations of antenatal depression with PTB and CRHBP with PTB.

4.3.3 Antenatal depression and serum (total) cortisol in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have higher serum cortisol. My findings do not support this hypotheses; I found no significant group difference and only a small sized effect (cases > controls) for the difference in serum cortisol. To my knowledge only one study in this field reports total cortisol: in this study (of a normal pregnant population) there was no correlation between plasma cortisol and BDI scores (Salacz et al., 2012). Serum cortisol provides a measure of total cortisol, in contrast to saliva cortisol, which provides a measure of free (biologically available) cortisol; however, serum and saliva cortisol are known to be correlated (Vining et al., 1983). Indeed, serum cortisol in this study was correlated with other saliva cortisol measures at baseline (for example, with awakening cortisol $r_p = .52$, $p = .001$, and diurnal cortisol (AUC) $r_p = .44$, $p = .011$) and furthermore at 32 weeks gestation (for example, with awakening cortisol $r_p = .64$, $p \leq .001$, and diurnal cortisol (AUC) $r_p = .63$, $p = .001$). Therefore the sample size may account for the lack of significant difference¹.

4.3.4 Antenatal depression and awakening cortisol in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have higher awakening cortisol at 25 and 32 weeks gestation. My findings support the hypothesis at 32 weeks but not at 25 weeks gestation.

To my knowledge only two published studies have measured awakening cortisol in pregnancy for women with operationally defined MDD at similar gestational ages. My finding of higher awakening cortisol at 32 weeks gestation was in contrast to both those studies, one of which found no significant difference (O'Keane et al., 2011), and the other found lower awakening

¹ Serum cortisol was only available for subjects under the 'main' protocol.

cortisol (O'Connor et al., 2013b) at 32 weeks gestation. However, these studies differed from the current one on a number of factors that may account for the differences between them. Firstly, although the study of O'Keane et al. was performed in the same setting as that described in this thesis, the cases and control groups differed in a number of socio-demographic or potentially confounding factors, for example 81% of cases were Caucasian which would appear to contrast with my cases group where 47% were white, 29% of cases were single in contrast to 53% of my cases group who were living alone, lastly awakening cortisol was measured in fewer participants, 33 controls v 13 cases in contrast to 40 controls v 26 cases in my study. Secondly, the study of O'Connor et al. cannot be compared so easily since the design was different. However it differed in setting as it was conducted in the USA and all the subjects were classed as 'psycho-socially at-risk' by dint of the population served by the recruiting clinic. The proportion of depressed versus non-depressed was lower than in my study, furthermore, the depressed group included diagnoses of MDD and depression not otherwise specified but no group comparison of symptom severity or of social demographic factors was presented.

No studies have measured awakening cortisol at 25 weeks gestation. However, awakening cortisol has been measured at different gestational ages e.g. later in pregnancy (35-39 weeks gestation), when no difference was found (Hellgren et al., 2013) and earlier in pregnancy at 20 weeks gestation when lower awakening cortisol was found (O'Connor et al., 2013b). My finding of increased awakening cortisol in the depressed group mirrors findings in depressed non-pregnant populations. Since so few studies are described in the current literature, my findings add to the body of evidence and highlight the preliminary nature of this line of research.

Interestingly, awakening cortisol was associated with both caseness and ethnicity in my study (see section 3.2.2.1.4 above); compared with white women, black and minority ethnic women had lower awakening cortisol, both overall and by caseness. Similar ethnic differences in the HPA axis have been reported in pregnant (Glynn et al., 2007, Suglia et al., 2010) and non-pregnant (Bennett et al., 2004) women.

4.3.5 Antenatal depression and evening cortisol in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have higher evening cortisol at 25 and 32 weeks gestation. My findings support the hypothesis at 32 weeks but not at 25 weeks gestation.

To my knowledge, only one study of operationally defined MDD in pregnancy has reported evening cortisol; higher evening cortisol ($n=46$) at 32 weeks gestation was reported in women with MDD (O'Keane et al., 2011). Similarly, one further study found a higher evening cortisol at mid gestation in those with high levels of self-rated depression symptoms (Peer et al., 2013). My finding corroborates and refines the small amount of evidence that currently exists, since a larger number of subjects are studied. Furthermore, it is the first replication of the findings of O'Keane et al.

4.3.6 Antenatal depression and diurnal cortisol in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have higher diurnal cortisol (AUC) at 25 and 32 weeks gestation. My findings support the hypothesis at 32 weeks but not at 25 weeks gestation.

To my knowledge, only one study of operationally defined MDD in pregnancy has measured diurnal cortisol (O'Connor et al., 2013b). In their study of 101 pregnant women at high psychosocial risk for developing depression, O'Connor et al. found that those with a diagnosis of depression had a flattened, less sharply declining diurnal pattern of cortisol secretion (defined by change in cortisol over time, rather than AUC) and overall, a higher cortisol level throughout the day both at twenty and thirty-two weeks gestation. Since the authors also report a lower awakening cortisol in the depressed group (see section 4.3.4 above), the above statement (overall, a higher cortisol level across the day) implies a higher evening cortisol level in the depressed group, however, a group comparison of evening cortisol was not presented. Although I have measured diurnal cortisol by different means (AUC) and my study design differs (see section 4.3.4 above), my findings appear to corroborate those of O'Connor et al at 32 weeks gestation, but not earlier; thus my findings represent the first replication of this work.

4.3.7 Antenatal depression and cortisol awakening response/post-awakening cortisol in pregnancy

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have higher CAR and post-awakening cortisol at 25 and 32 weeks gestation. My findings do not support the hypothesis. In contrast, I found that compared to non-depressed controls, women with MDD in pregnancy had a blunted cortisol awakening response (AUCi), but

no difference in AUCg at 32 weeks gestation. There was no group difference in either measure of post-awakening cortisol at 25 weeks gestation.

To my knowledge, only two studies of operationally defined MDD in pregnancy have assessed CAR and post-awakening cortisol and neither study found a difference between depressed and non-depressed groups. However, both studies differed from that described in this thesis in design or measures: one study (n=108) measured CAR and post-awakening cortisol (AUCi and AUCg) later in pregnancy, between 35 and 39 weeks gestation in three groups (depressed in pregnancy, history of depression, no depression) (Hellgren et al., 2013) and the second study (n=101) used the change from awakening cortisol to 45 minutes post-awakening to represent CAR at 20 and 32 weeks gestation in women at high risk of depression, either with or without MDD in pregnancy (O'Connor et al., 2013b). Similarly, studies using high v low levels of self-rated depression symptoms found no differences in CAR or post-awakening cortisol (Peer et al., 2013, Shea et al., 2007). Thus, although the number of subjects providing data for CAR in this study was small, the finding of a blunted cortisol awakening response (AUCi) at 32 weeks gestation should be considered as an extension of current knowledge rather than conflicting results, and is thus a novel finding.

4.3.8 Summary of antenatal depression and HPA axis in pregnancy

Overall, my findings corroborate those of the majority of the small amount of literature in this field; that antenatal depression is associated with overactivity in the HPA axis in the third trimester of pregnancy. They also mirror HPA axis findings from studies of MDD in non-pregnant populations. Moreover, my findings extend the current knowledge either by more detailed HPA axis assessment or a larger sample size; at the same time, potential confounding factors are controlled for in the analyses. There is little in the published literature on antenatal depression and HPA axis at 25 weeks, and although I found no group differences in HPA axis measures at this gestational age, I did find the same pattern of differences in the cortisol measures at baseline as at 32 weeks gestation. Although, there was no statistically significant difference in these measures between cases and controls at baseline, there were fewer subjects with cortisol data available at this assessment. Furthermore, there were statistically significant and strong positive correlations between 25 and 32 weeks for awakening cortisol ($r_p = .62$, $p < .001$), diurnal cortisol ($r_p = .55$, $p = .003$) and post-awakening cortisol (AUCg) ($r_p = .77$, $p < .001$), and a statistical trend for a moderate correlation for evening cortisol ($r_s = .31$, $p = .09$) but none for CAR (AUCi) ($r_p = .37$,

$p = .14$). One explanation could be that there was insufficient power to show group differences in the measures at baseline; alternatively, my findings could be a true reflection of no group difference at this gestational age.

In addition to the paucity of literature on MDD in pregnancy and maternal HPA axis, there is little literature regarding the effect of other forms of stress or anxiety in pregnancy on the maternal HPA axis. This is surprising since there is much literature hypothesising overactivity of maternal HPA axis as a biological mechanism underlying developmental programming. It appears that this first step in unravelling the mechanisms between suboptimal maternal mental health and developmental programming has been somewhat understudied, perhaps because animal studies have already suggested the link convincingly; nonetheless one cannot assume that the same will apply in humans. Thus, confirmation of overactivity of maternal HPA axis in antenatal depression is an important addition to the literature in the emerging field of research into the mechanisms of developmental programming in humans.

Moreover, and critically, overactivity of maternal HPA axis in antenatal depression is of clinical relevance. There is already a mandate, in the UK, to screen for and actively treat depression in pregnancy because of its known association with a range of adverse obstetric and offspring outcomes. However, my findings reinforce the notion that antenatal depression is also associated with altered maternal physiology in a system (HPA axis) that is not simply a vital homeostatic one, but one that is also central to the maintenance of pregnancy, timing of delivery, foetal maturation and foetal development. Thus, my findings strengthen the argument for this mandate; clinicians responsible for treating MDD can feel more confident that MDD should be managed both in a timely fashion and actively, because not to do so has the clear potential for adverse consequences. Moreover, the findings add evidence to the clinician's armamentarium in the difficult arena of clinical decision-making when evaluating the risks and benefits of using antidepressant medication in pregnancy.

4.4 Antenatal depression and obstetric outcome

I hypothesised that compared with non-depressed pregnant women those with antenatal depression would have a higher rate of PTB, a greater proportion of LBW and SGA babies, a shorter length of gestation and babies of lower birth weight.

The findings demonstrate, in women with spontaneous onset of labour at term, that those with antenatal depression had a shorter length of gestation (mean = 4.6 days) than women without antenatal depression; this difference represented a small to medium sized effect. The finding was reduced to a trend level of significance when marital status was taken into account, however marital status is an important risk factor for MDD, thus the effect of marital status on gestational age at birth cannot be disentangled from the effect of caseness. Because of the small number of instances, the potential effect of antidepressant medication was not taken into account although the difference in length of gestation was not accounted for by any other potentially confounding factors. Lastly, group differences were not apparent in PTB rate, birth weight, low birth weight babies or small for gestational age babies.

Information regarding the effect of operationally defined MDD in pregnancy on obstetric outcome is limited (as described in section 1.2.4.6.2 above). Only six studies included in the two (relatively recent) meta-analyses of this area were of operationally defined MDD (5 of PTB, 2 of LBW, 1 of IUGR, 2 of length of gestation and 4 of birth weight) (Diego et al., 2009, Field et al., 2010, Field et al., 2006a, Suri et al., 2007, Wisner et al., 2009, Rahman et al., 2004). To my knowledge, only two other relevant studies are not included in these meta-analyses: Firstly, O'Keane et al. reported a shorter length of gestation in a depressed, compared with a non-depressed group in their case control study (n=54) (O'Keane et al., 2011). Secondly, in their population-based cross-sectional study of all singleton births in Finland between 2002 and 2010, Raisanen et al. reported that compared to women without depression in pregnancy, those with physician-diagnosed ICD-10 depression had increased odds (very similar values to those reported in the 2 meta-analyses) of PTB, LBW and SGA (n = 511,938); length of gestation and birth weight were not reported in this study (Raisanen et al., 2014). The evidence for the association of MDD in pregnancy with increased rates of PTB, LBW and SGA is compelling. There are fewer publications on length of gestation (only 1 of the three studies found a significant difference (O'Keane et al., 2011), but on closer scrutiny, the two studies finding no difference reported gestational age at birth for all

deliveries, rather than those with spontaneous onset of labour (Diego et al., 2009, Suri et al., 2007)).

4.4.1 Summary of antenatal depression and obstetric outcome

In summary, my finding of shorter length of gestation in women with MDD in pregnancy compared with women without MDD mirrors the results of the only other similar study (O'Keane et al., 2011). Crucially, any association of length of gestation with MDD will be masked if all deliveries, rather than only those with a spontaneous onset of labour are considered; thus the two other studies reporting on this phenomenon are not comparable, nor are they informative regarding this measure. An emerging literature is suggestive of subtle impairments in brain development and cognitive ability in addition to respiratory health and increased risk of SIDS for children born at lower gestational ages, within the 'at term' range. Thus even small differences in length of gestation may have a clinically relevant effect on offspring. Therefore, since MDD in pregnancy is common, an association with shorter length of gestation may be of importance for public health.

I found no difference in PTB, LBW, SGA or birth weight. However, the number of subjects in my study was small compared with studies showing an effect (which have subject numbers in the hundreds or more), and thus lacked the power to show an effect when the relative risk is of the order of magnitude suggested in the literature.

4.5 Antenatal depression, antenatal HPA axis and obstetric outcome

I hypothesised that obstetric outcome would be associated with maternal antenatal HPA axis measures. However, I found no correlation between gestational age at birth (in spontaneous deliveries at term) with the antenatal HPA axis measures that differed between cases and controls. Furthermore, I hypothesised that the antenatal HPA axis would be a moderator or mediator in the pathway from AND to pregnancy outcome but I found no evidence to support this. Associations between HPA axis and obstetric outcomes are reviewed in section 1.4.4.4. In brief, fairly consistent links between CRH and length of gestation and/or PTB are described in the literature, although one large study of preterm birth found no association with CRH (adjusted for potential confounding factors) measured at 24-26 weeks gestation (Kramer et al., 2013). Antenatal cortisol and CAR have also been associated with adverse obstetric outcomes. To my

knowledge there are no studies of the role of maternal HPA axis in the association between operationally defined MDD in pregnancy and obstetric outcomes, although the stress in pregnancy literature contains some findings of associations between stress, CRH and PTB (Himes and Simhan, 2011, Mancuso et al., 2004, Rich-Edwards and Grizzard, 2005, Sandman et al., 1997, Kramer et al., 2009).

Although associations of antenatal HPA axis and obstetric outcomes have been described in the literature, the samples sizes used are larger than mine; thus my study may have lacked the power to show any such associations. Alternative explanations might be that pathways to suboptimal obstetric outcomes may be more complex, involving other biological systems that have not been assessed in this study.

As discussed in section 4.3.8 above, my findings are also of clinical relevance to obstetric clinicians (obstetricians, midwives and GPs) since confirmation of an association between antenatal depression and altered maternal physiology in a system (HPA axis) that is not simply a vital homeostatic one, but one that is also central to the maintenance of pregnancy, timing of delivery, foetal maturation and foetal development and obstetric outcome is of clinical relevance despite the absence of a finding in this study of an association between the combination of all three factors (antenatal depression, maternal antenatal HPA axis and obstetric outcome).

4.6 Antenatal depression and offspring HPA axis at 8 weeks and 1 year postnatal

I hypothesised that compared with infants not exposed to depression *in utero* those who had been exposed would have greater cortisol reactivity, indexed both by cortisol response to immunizations and higher cortisol after the immunizations, at 8 weeks and 1 year of age. Furthermore, that they would have higher basal (awakening and evening) cortisol at both ages.

4.6.1 Cortisol reactivity

The findings demonstrate that compared with 1-year-old infants not exposed to depression *in utero*, those who had been exposed had a higher cortisol level after the immunization. In 8-week-old infants a similar pattern of difference was apparent but reached only a trend level for statistical

significance; however, the size of the effect was medium, as it was at 1 year postnatal, and the 95% confidence intervals did not cross zero. Univariate analyses showed that delta cortisol at both postnatal time points was higher in infants exposed to depression *in utero* compared with infants who were not so exposed; however, this difference was not statistically significant at either time, although it represented a small to medium sized effect at both assessments.

Furthermore, in 8-week-old infants, immunization was associated with a statistically significant effect (increase) on cortisol in both groups. This effect was greater in infants who had been exposed to depression *in utero* than in those not so exposed ($r = .52$ v $.37$ respectively). Lastly, in 1-year-old infants, immunization was associated with a moderate sized effect (increase) on cortisol. This was at a trend level of statistical significance in infants who had been exposed to depression *in utero*, but there was no effect in infants not exposed to depression *in utero* ($r = .30$ v $.04$ respectively).

4.6.2 Basal cortisol

The findings demonstrate, in 1-year-old infants, that compared with infants not exposed to depression *in utero* those who were exposed had higher evening cortisol and the difference represented a medium sized effect; however, no such group difference was apparent in 8-week-old infants. There was no significant group difference in awakening cortisol in 8-week or 1-year-old infants.

A small number of prospective studies have examined the effect of self-rated symptoms of depression on offspring HPA axis (as described in section 1.5.3.2.1 above). Overall, the findings suggest that, as for the broader concept of prenatal stress, symptoms of depression in pregnancy may have a programming effect on offspring HPA axis; this has been evaluated in a range of offspring ages from newborn to adolescence and by either basal cortisol or cortisol reactivity to stress. To my knowledge only one other prospective study of HPA axis in offspring who were exposed to operationally defined MDD *in utero* has been published. An association between antenatal depression (and depression comorbid with anxiety) and higher neonatal basal cortisol was reported, but interpretation of this study is hampered by a narrow assessment of offspring HPA axis and number of important methodological limitations (Field et al., 2010). Therefore, my findings are in line with the existing literature; moreover, they represent an important addition, being the first to report persisting heightened HPA axis function, in 8 week-old and 1-year-old

infants, and increased basal HPA axis activity in 1-year-old infants exposed to operationally defined MDD *in utero*. Consequently this is an important addition both to the developmental programming and antenatal depression literature.

In contrast to my findings in 1-year-old infants, 8-week-old infants exposed to depression *in utero* showed no significant difference in basal cortisol, (awakening or evening) compared to infants who were not exposed. In general an association between basal cortisol in offspring and antenatal depression has been reported. For example higher urinary cortisol in neonates who were exposed to depression or a high level of depressive symptoms in pregnancy (Diego et al., 2004, Field et al., 2010, Lundy et al., 1999) or decreased CAR and flatter diurnal cortisol slope in 15-year-old offspring who were exposed to high levels of symptoms both of depression and of anxiety in pregnancy (O'Donnell et al., 2013). The absence of such an association at 8 weeks postnatal appears to be in contrast to the findings of others; however, it is not directly comparable as cortisol in the above studies was measured at different offspring ages. The emergence of a difference at 1 year postnatal may reflect the dynamics of HPA axis development over time. Alternatively it may be related to other unmeasured factors; interestingly it did not appear to be accounted for by further exposure to maternal depression in the postnatal period. The lack of difference in awakening cortisol together with higher evening cortisol in 1-year-olds exposed to depression *in utero* can be likened to the finding of O'Donnell of a flattened diurnal slope of cortisol secretion in 15 year olds and represents an altered set point of offspring HPA axis.

Moreover, my finding of an effect of immunization on infant cortisol at 1 year postnatal in infants who were exposed to depression *in utero* (albeit at a trend level of statistical significance), but no effect in infants not so exposed adds to the broader literature on cortisol response to pain in infants. Cortisol response to a variety of stressors, from age 0-18 years, has been reviewed (Jansen et al., 2010, Gunnar et al., 2009). Pain is fairly consistently shown to produce an increase in cortisol in studies of children up to 6 months of age but seldom beyond that. However, to my knowledge, there are no published studies comparing cortisol response to pain in infants of women with high v low levels of psychological symptoms or of stress in pregnancy. A cortisol response to immunization in the infants of controls at 8 weeks postnatal, which is no longer present at 1 year of age, is consistent with the published literature. However, the finding of an effect of immunization on cortisol of 1-year-old infants exposed to depression *in utero* is

seemingly out of kilter with the existing literature. However, the phenomenon has not been studied in infants exposed to an adverse intrauterine environment such as in this study. This finding suggests that the set point of the HPA axis, which does not normally mount a response to immunization much beyond the age of 6 months, is altered in infants who were exposed to MDD *in utero*, and is thus a novel finding.

4.6.3 Summary of antenatal depression and offspring HPA axis at 8 weeks and 1 year postnatal

In summary my findings demonstrate an association between antenatal depression and infant HPA axis function and activity in the first year of life. This is in keeping with the broader human and animal literature on the effect of stress in pregnancy and developmental programming and extends and refines the knowledge base on the specific effects of antenatal depression.

Confirmation of overactivity and altered set point of offspring HPA axis in infants exposed to depression *in utero* is an important addition to the literature in the emerging field of research into developmental programming in humans. It further strengthens the mandate to screen for and actively treat depression in pregnancy (discussed in section 4.3.8 and 4.5 above). My findings not only reinforce the notion that antenatal depression is associated with altered maternal physiology in pregnancy (discussed in 4.3.8 above), but also that occurrence of MDD in pregnancy is associated with alterations in the same vital system in the offspring. By extension, these findings indicate that since the HPA axis is altered in infants exposed to depression *in utero*, adverse health outcomes may be associated and so the findings are of clinical relevance. Available evidence affords little doubt that exposure to an adverse early environment is associated with cardiovascular disease, metabolic syndrome and other unfavourable health outcomes in adulthood and it is hypothesized that alterations in offspring HPA axis mediate these effects. Should this be the case, individuals who were exposed to depression *in utero* may benefit from clinical monitoring in order to identify emerging health problems, which thus may be prevented or ameliorated before the onset of symptomatic disorders in adulthood. Moreover, an opportunity for earlier intervention would also be afforded: animal and human literature suggest that alterations in offspring HPA axis or other programming effects may be reversed by early care maternal care or environment (Zhang et al., 2013).

4.7 Antenatal depression, antenatal HPA axis and infant HPA axis at 8 weeks and 1 year postnatal

I hypothesised that maternal antenatal HPA axis measures would be correlated with offspring HPA axis measures at 8 weeks and at 1 year postnatal. The findings demonstrate that at 1 year postnatal there was a statistically significant negative correlation between both maternal antenatal awakening cortisol and diurnal cortisol (AUC) at 32 weeks gestation and infant cortisol before the immunization at 1 year postnatal; the strength of the associations were moderate to strong. Furthermore there was a statistically significant positive correlation between both maternal antenatal evening cortisol and diurnal cortisol (AUC) at 32 weeks gestation and infant delta cortisol at 1 year postnatal; again, the strength of the associations were moderate to strong. Lastly, there was a trend level of statistical significance for a positive correlation between maternal antenatal evening cortisol at 32 weeks gestation and infant cortisol after the immunization at 1 year postnatal; the strength of the association was moderate. There were no significant correlations between maternal antenatal HPA axis measures and offspring HPA axis measures at 8 weeks postnatal. These findings demonstrate that a relationship exists between maternal antenatal cortisol and infant HPA axis and furthermore, demonstrate a degree of continuity, since the direction of the correlation corresponded with the direction of the group difference (between cases and controls) in maternal antenatal cortisol in relation to that found in the infant cortisol.

Secondly, I hypothesised that maternal antenatal HPA axis measures would moderate or mediate the pathway from antenatal depression to offspring HPA axis activity at 8 weeks and at 1 year postnatal. The findings demonstrate that maternal diurnal cortisol at 32 weeks gestation moderated the effect of caseness on infant delta cortisol at 8 weeks of age. To expand further, compared with infants who were not exposed to depression *in utero*, infants of women so exposed had significantly higher delta cortisol when antenatal diurnal cortisol was ≥ 6938.6 nmol/ml² and I have already shown that diurnal cortisol at 32 weeks gestation was higher in depressed cases than non-depressed controls. This finding demonstrates that a relationship

exists between antenatal depression, antenatal cortisol and infant HPA axis function, although no evidence of mediation was found.

I found no evidence that the maternal antenatal HPA axis measures that differed between cases and controls were moderating or mediating the effect of caseness on any other infant cortisol measures at 8 weeks or at 1 year postnatal. The lack of evidence for mediating effects may well be due to the relatively small sample sizes for the infant cortisol measures (ranging from 43 at the 1 year postnatal immunizations to 61 for basal cortisol at 8 weeks postnatal). Given these sample sizes, the effect size for both the 'a' and the 'b' paths in the mediation model (see section 2.5.1.2 above) would both need to be relatively large to have the power to find a positive result. Sample sizes of approximately 400 to 500 would be required in order to avoid type II error if the effect size was small for both paths, and in the region of 100-150 for more moderate sized effects (Fritz and Mackinnon, 2007). Alternatively, the pathways to infant HPA axis may be more complex, involving other biological systems that have not been assessed in this study.

There is some evidence that a high (versus low) level of maternal cortisol in pregnancy is associated with cortisol response to stress in offspring (Davis et al., 2011b, Gutteling et al., 2004, Gutteling et al., 2005). Furthermore, a high (versus low) level of amniotic fluid cortisol (an index of direct *in utero* exposure to cortisol) was shown to be associated with cortisol response to stress in offspring (O'Connor et al., 2013a). Indeed, my findings of correlation between some of the maternal antenatal and infant HPA axis measures are in keeping with these studies. Furthermore, on balance, the evidence in the literature, outlined in section 1.4.5 above and from this current study (section 4.3.8 above) suggests that the maternal HPA axis is overactive in depression in pregnancy. However, to my knowledge there are no published studies of the combination of these three factors, i.e. antenatal depression, maternal antenatal cortisol and infant cortisol. Indeed, that the effect of caseness on infant delta cortisol at 8 weeks postnatal was moderated by maternal antenatal diurnal cortisol (AUC) further strengthens the case for the existence of a mechanistic pathway from maternal antenatal HPA axis to the programming of offspring HPA axis, is a novel finding and adds to the emerging literature on mechanisms of developmental programming in humans.

4.8 Methodological strengths and limitations

This study had a number of important strengths, for example the prospective design, diagnoses obtained through semi-structured clinical interview and a relatively broad assessment of HPA axis in mothers and their infants over several time points. Furthermore, much information was collected in order to examine for potential confounding factors. This included socio-demographic factors for both parents, obstetric history, obstetric risk factors, maternal antenatal physical health, health behaviours, medications and mental health. Moreover, these factors were examined in relation to infant HPA axis measures with the addition of exposure of the foetus to antenatal symptoms of anxiety (much existing literature on developmental programming examines the effect of antenatal symptoms of anxiety), exposure to maternal postnatal depression (this may affect the handling of the infant and, in turn, their cortisol), concurrent (postnatal) maternal HPA axis measures (cortisol is secreted in breast milk, thus may account for cortisol levels in breastfed infants (Benjamin Neelon et al., 2015)), neonatal neurobehaviour, infant health and medication and infant development (cognitive, language and motor). Despite this there are a number of methodological limitations, which are discussed in the following section.

The first limitation was the sample size. With 82 participants the sample size was unlikely to be adequate to demonstrate differences in outcomes where the effect sizes between groups was potentially small, for example the categorical obstetric outcomes, or to demonstrate anything other than large indirect (mediation) effects, accordingly the lack of positive findings where hypothesised could reflect type II error as opposed to no true difference. Furthermore, the sample size diminished further over the course of the study, which was approximately 18 months per participant. Subject retention is an inevitable issue in longitudinal studies. However, this issue is particularly pertinent in longitudinal studies that begin in pregnancy, since pregnancy (even when strongly desired) and the first postnatal year are periods of incredible change and inherent stress for parents. This point, compounded by the fact that pregnant women frequently have other young children at home (55% of participants in this study), has the potential to make subject retention even more of a challenge.

Secondly, subjects were drawn from the catchment population of Kings College Hospital and South London and Maudsley NHS Trusts. This includes particularly wide-ranging diversity in

socio-economic status and ethnicity. Although there were no significant differences in these factors between women who participated and those who refused or were not contactable, they may not reflect the wider UK population, which would thus limit the generalizability of the results.

Third, the change between 'pilot' and 'main' protocols meant that there were fewer observations in some variables. Furthermore, more researchers participated in assessments and data collection. Steps were taken to minimise the impact of this since I personally reviewed all diagnostic interviews with the researcher who had performed them and set up a procedure for monitoring and reviewing the quality of assessments.

Fourth, although controls were free from lifetime psychiatric disorder, this was not so for cases. In order to recruit sufficient numbers of cases, those with a past history of psychiatric disorder (other than bipolar affective disorder or psychosis) were included. Clearly this was not ideal but a history of other psychiatric disorders in cases of depression is not uncommon and recruitment of a 'pure' depression group had to be balanced against recruitment of sufficient numbers of cases.

Fifth, although cases were free from antidepressant medication at baseline, a small proportion took antidepressants later in pregnancy. The potential effect of antidepressant use in pregnancy on obstetric outcome could not be quantified because of the infrequency of this potential confounding factor.

Lastly, despite their continued involvement in the study, not all subjects and their infants provided usable saliva samples for measurement of cortisol. Other than the saliva samples obtained from infants on the day of immunization (when a researcher was present) there was no control on adherence to the sampling protocol or accurate recordings of timings. However, steps were taken to minimise errors by clear demonstration of the correct procedures including emphasis on the importance of the sampling protocol and record of sample timings and the provision of written instructions as a reminder. Although samples that were not acquired within our stipulated times were not used in the data analyses, this did not guarantee the accuracy of the data which did appear to meet the requirements. Measures to control these factors, such as actimetry units (to show wake time) and medication event monitoring (MEM) systems to show sampling times, were not employed.

4.9 Future directions

Since the thesis describes some findings that are first reports, replication is warranted. A larger sample size would be required, taking the issue of retention rates into account, to ensure adequate power to detect some of the effects, and particularly to test for mediation. Furthermore it would be beneficial to include an arm to examine the effects of exposure to antidepressant medication *in utero* on offspring HPA axis. A measure of the quality of postnatal caregiving would also be desirable in order to control for offspring postnatal environment (O'Connor et al., 2013a).

A next step would be to study the potential molecular mechanisms of the developmental programming effects of depression in pregnancy, for example, epigenetic studies examining genes related to the HPA axis and 11 β -HSD-2. A small literature now exists although this line of research is in its infancy and at the current time is confined to symptoms of, rather than diagnoses of depression (Braithwaite et al., 2015, Oberlander et al., 2008).

Clearly further study is required to determine if there is longer term persistence of HPA axis change in the offspring; although longer term changes are suggested, at least into adolescence in humans (O'Donnell et al., 2013, Vedhara et al., 2012), and in animals, even beyond that to the next generation (Schoppe et al., 2012).

Furthermore, and related to this issue is to elucidate any associated changes in offspring health and behaviour. Contingent on this, the potential for interventions, for example environmental or pharmaceutical, to reverse or ameliorate the effects of altered offspring HPA axis, may be warranted.

4.10 Conclusions

In summary I have demonstrated a novel finding of altered HPA axis function and activity that persists over the first year of life in infants who were exposed to major depression *in utero* compared to infants who were not so exposed. Although no evidence of mediation was found, I have shown that a relationship exists between antenatal depression, maternal antenatal HPA axis and infant HPA axis in the first year of life. This relationship was evidenced by significant correlation between some infant and maternal HPA axis measures; and furthermore, that a group difference in delta cortisol in 8-week-old infants was only apparent when maternal antenatal diurnal cortisol was high (as it was in women with antenatal depression compared with controls). Furthermore, I have added to the small body of current literature on major depression in pregnancy, maternal antenatal HPA axis and obstetric outcomes, demonstrating an overactive maternal HPA axis and shorter length of gestation in women with major depression in pregnancy compared to non-depressed pregnant women. Overall, these findings add to the important literature on developmental programming and moreover, have potential implications for clinical practice and public health.

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Appendix A Differences between pilot and main protocol

Table 47: Comparison of 'pilot' and 'main' protocols

	Pilot protocol	Main protocol
Inclusion criteria		
Maternal age	<ul style="list-style-type: none"> 18 to 40 years 	<ul style="list-style-type: none"> 18 to 45 years
Cases diagnosis	<ul style="list-style-type: none"> MDD at baseline 	<ul style="list-style-type: none"> MDD in pregnancy at or prior to baseline
Exclusion criteria		
Medication	<ul style="list-style-type: none"> Regular medication 	<ul style="list-style-type: none"> Regular medication was not an exclusion criterion
Diagnosis for cases	<ul style="list-style-type: none"> Any axis 1 diagnosis other than anxiety disorders 	<ul style="list-style-type: none"> Psychosis or bipolar affective disorder only
Recruitment process – all subjects		
	<ul style="list-style-type: none"> No recruitment script No concise colour pamphlet 	<ul style="list-style-type: none"> Recruitment script Concise colour pamphlet
Recruitment process for controls		
	<ul style="list-style-type: none"> Screened for inclusion and exclusion criteria at initial approach. 	<ul style="list-style-type: none"> Screened for inclusion and exclusion criteria at second (telephone) contact.
Recruitment process for cases		
	<ul style="list-style-type: none"> At this time, most referrals for depression were not seen in the Perinatal Psychiatry clinic. As a psychiatrist, and the only person recruiting subjects throughout the pilot study, I made telephone contact with women who were potentially eligible to participate as cases. During the call, having established that they were currently depressed and were happy to have been referred by their GP, I invited them to see me for a clinical assessment, following which and if they were eligible, I invited them to participate. Women who required clinical follow-up were referred on to my colleagues within the department. In cases where it was not possible to contact the woman by telephone, no different approaches were pursued. 	<ul style="list-style-type: none"> The decision about contacting a woman as a case was made at the level of the clinical referrals meeting and contact about the study was made by researchers who were not part of the clinical team. If it was not possible to make telephone contact, a letter was sent to the woman and the researcher provided an appointment to discuss the research at the woman's home. If that appointment was not attended an invitation to make contact was left at the woman's home.

Procedures & measures		
Characteristics		
	<ul style="list-style-type: none">• Less information was obtained on characteristics of the sample, for example paternal characteristics, maternal PRN medication.• Premorbid IQ was not assessed, although this was done subsequently where possible (n= 12).• At 8 weeks & 1 year postnatal less information was obtained on infant characteristics, e.g. feeding problems, medical appointments.	
Psychiatric assessment		
	<ul style="list-style-type: none">• Self-rated symptom scores were not obtained at 32/40	<ul style="list-style-type: none">• Self-rated symptom scores were obtained at 32/40
HPA 1		
	<ul style="list-style-type: none">• Serum cortisol was not measured.	<ul style="list-style-type: none">• Serum cortisol was measured
HPA 2		
	<ul style="list-style-type: none">• Saliva cortisol was not obtained at baseline	<ul style="list-style-type: none">• Saliva cortisol was obtained at baseline.
HPA 3		
	<ul style="list-style-type: none">• Awakening and evening samples only (not CAR, or midday samples) were obtained at 32 weeks gestation, 8 weeks and one year postnatal	<ul style="list-style-type: none">• Samples for assessment of CAR and midday was assessed at each time point.
HPA 4		
	<ul style="list-style-type: none">• I performed all blood processing	<ul style="list-style-type: none">• Most blood processing was performed by laboratory staff at KCH

Appendix B Protocol for antenatal interview

Subject ID (no.-initials) -

Researcher's Initials

DATE - -



P.R.A.M.
Psychiatry
Research and
Motherhood

Psychiatry Research & Motherhood (PRAM) Study

Antenatal interview booklet

Demographic details

Medication record and Medical history

Obstetric history

Smoking history, tea, coffee and alcohol consumption

DEMOGRAPHIC DETAILS

I'd like to start by asking a few background questions about you.

1. What is your date of birth?

--	--	--	--	--	--

2. What ethnic group do you consider you belong to?

White

- 01 British
- 02 Irish
- 03 Any other White background

Please specify.....

Black or Black British

- 12 Caribbean
- 13 African
- 14 Any other Black background

Please specify.....

Mixed

- 04 White and Black Caribbean
- 05 White and Black African
- 06 White and Asian
- 07 Any other Mixed background

Please specify.....

Chinese or other ethnic group

- 15 Chinese
- 16 Any other ethnic group

Please specify.....

Asian or Asian British

- 08 Indian
- 09 Pakistani
- 10 Bangladeshi
- 11 Any other Asian background

Please specify.....

--	--

3. Where were you born? Where were you brought up?

- 1 = Born in UK / majority of childhood in UK
- 2 = Born in UK / majority of childhood elsewhere
- 3 = Born elsewhere / majority of childhood in UK
- 4 = Born elsewhere / majority of childhood elsewhere

--

If majority of childhood elsewhere: Did you have 9 or more years in an English-speaking school?

0 = No 1 = Yes

--

If born elsewhere: Did you have at least 4 years in an English-speaking secondary school?

0 = No 1 = Yes

--

4. What is your first language? 1 = English 2 = Other (*specify*) _____

--

5. Are you married or living with someone? *If no:* Do you have a boyfriend or partner?

- 1 = Single (no partner)
- 2 = Single (with partner)
- 3 = Cohabiting
- 4 = Married

--

5.a. If has partner: How long have you been together? (*years; if <1 year enter 00*)

--	--

DEMOGRAPHIC DETAILS

I'd like to start by asking a few background questions about you.

1. What is your date of birth?

--	--	--	--	--	--

2. What ethnic group do you consider you belong to?

White

- 01 British
- 02 Irish
- 03 Any other White background

Please specify.....

Black or Black British

- 12 Caribbean
- 13 African
- 14 Any other Black background

Please specify.....

Mixed

- 04 White and Black Caribbean
- 05 White and Black African
- 06 White and Asian
- 07 Any other Mixed background

Please specify.....

Chinese or other ethnic group

- 15 Chinese
- 16 Any other ethnic group

Please specify.....

Asian or Asian British

- 08 Indian
- 09 Pakistani
- 10 Bangladeshi
- 11 Any other Asian background

Please specify.....

--	--

3. Where were you born? Where were you brought up?

- 1 = Born in UK / majority of childhood in UK
- 2 = Born in UK / majority of childhood elsewhere
- 3 = Born elsewhere / majority of childhood in UK
- 4 = Born elsewhere / majority of childhood elsewhere

--

If majority of childhood elsewhere: Did you have 9 or more years in an English-speaking school?

0 = No 1 = Yes

--

If born elsewhere: Did you have at least 4 years in an English-speaking secondary school?

0 = No 1 = Yes

--

4. What is your first language? 1 = English 2 = Other (*specify*) _____

--

5. Are you married or living with someone? *If no:* Do you have a boyfriend or partner?

- 1 = Single (no partner)
- 2 = Single (with partner)
- 3 = Cohabiting
- 4 = Married

--

5.a. If has partner: How long have you been together? (*years; if <1 year enter 00*)

--	--

5.b. Is your current partner the father of the baby? 0 = No 1 = Yes

☐

5.c. What ethnic group do you consider the father of the baby belongs to?

White

- 01 British
- 02 Irish
- 03 Any other White background

Please specify.....

Black or Black British

- 12 Caribbean
- 13 African
- 14 Any other Black background

Please specify.....

Mixed

- 04 White and Black Caribbean
- 05 White and Black African
- 06 White and Asian
- 07 Any other Mixed background

Please specify.....

Chinese or other ethnic group

- 15 Chinese
- 16 Any other ethnic group

Please specify.....

Asian or Asian British

- 08 Indian
- 09 Pakistani
- 10 Bangladeshi
- 11 Any other Asian background

Please specify.....

☐☐

6. What level of educational qualifications do you have?

- | | |
|------------------------------|---|
| 0 = No formal qualifications | 3 = Vocational/ college (B.Tecs/NVQs etc) |
| 1 = GCSEs/ O levels | 4 = Higher education (degree/diploma) |
| 2 = A-levels | |

☐

7. Are you working at the moment?

- | | |
|----------------------------------|----------------------------------|
| 1 = Employed, working | 4 = Unemployed/ full-time mother |
| 2 = Employed, on maternity leave | 5 = Student |
| 3 = Employed, on sick leave | 6 = Other |

☐

Establish occupational status. If not currently employed, ask about last job.

7.a. Do (did) you work as an employee or are (were) you self-employed?

- 1 = Employee
- 2 = Self-employed with employees
- 3 = Self-employed / freelance without employees

☐

7.b. *For employees:* How many people work (worked) for your employer at the place where you work (worked)?

- 1 = 1 to 24 2 = 25 or more (not for data entry)

☐

For self-employed: How many people do you employ (did you employ)?

- 1 = 1 to 24 2 = 25 or more (not for data entry)

☐

7.c. Do (did) you supervise other employees? 0 = No 1 = Yes (not for data entry)

☐

7.d. What is (was) your occupation? _____

- | | |
|-------------------------------------|--------------------------------|
| 1 = Modern professional | 5 = Routine manual and service |
| 2 = Clerical and intermediate | 6 = Middle or junior managers |
| 3 = Senior manager or administrator | 7 = Traditional professional |
| 4 = Technical and craft | |

☐

8. *Ask about cohabiting partner:* Does your husband/partner work?

☐

- | | |
|----------------------------------|-------------|
| 1 = Employed, working | 4 = Student |
| 2 = Employed, on sick leave | 5 = Other |
| 3 = Unemployed/ full-time father | |

Establish occupational status. If not currently employed, ask about last job.

8.a. Does (did) he work as an employee or is (was) he self-employed?

- | |
|---|
| 1 = Employee |
| 2 = Self-employed with employees |
| 3 = Self-employed / freelance without employees |

☐

8.b. *For employees:* how many people work (worked) for his employer at the place where he works (worked)?

- | | |
|-------------|-------------------------------------|
| 1 = 1 to 24 | 2 = 25 or more (not for data entry) |
|-------------|-------------------------------------|

☐

For self-employed: how many people does he employ (did he employ).

- | | |
|-------------|-------------------------------------|
| 1 = 1 to 24 | 2 = 25 or more (not for data entry) |
|-------------|-------------------------------------|

☐

8.c. Does (did) he supervise other employees? 0 = No 1 = Yes (not for data entry)

☐

8.d. What is (was) his occupation? _____

- | | |
|-------------------------------------|--------------------------------|
| 1 = Modern professional | 5 = Routine manual and service |
| 2 = Clerical and intermediate | 6 = Middle or junior managers |
| 3 = Senior manager or administrator | 7 = Traditional professional |
| 4 = Technical and craft | |

☐

CURRENT MEDICATION

1. Are you currently taking any *regular* medications, including oral medication (including dietary supplement), creams and inhalers?

0 = No 1 = Yes

☐

- a) Drug Name _____
i) Dose (mg/day) _____
ii) Start date (month / year) _____ / _____
iii) Route of administration: _____
- b) Drug Name _____
i) Dose (mg/day) _____
ii) Start date (month / year) _____ / _____
iii) Route of administration: _____
- c) Drug Name _____
i) Dose (mg/day) _____
ii) Start date (month / year) _____ / _____
iii) Route of administration: _____
- d) Record any other drugs here in the same way

Are you currently taking any *as required* medications, including oral medication (including dietary supplement), creams and inhalers?

0 = No 1 = Yes

☐

- a) Drug Name _____
i) Dose (mg/day) _____
ii) Start date (month / year) _____ / _____
iii) Route of administration: _____
- b) Drug Name _____
i) Dose (mg/day) _____
ii) Start date (month / year) _____ / _____
iii) Route of administration: _____
- c) Drug Name _____
i) Dose (mg/day) _____
ii) Start date (month / year) _____ / _____
iii) Route of administration: _____
- d) Record any other drugs here in the same way

2. Have you taken folic acid before or during this pregnancy?

0 = No 1 = Yes

☐

Start date (month / year)

End date (month / year)

MEDICAL HISTORY

1. I would like to ask you about any medical problems you might have.

Do you have any chronic medical conditions?

0 = No 1 = Yes

☐

Comments:

I would like to go through a list with you to check if you have any of the following disorders?

Check individually through each system below:

a) Respiratory or lung disease
(e.g. asthma, Chronic obstructive airways disease)

0 = No 1 = Yes

☐

Comments:

b) Endocrine disorder (e.g. thyroid disease, Cushing's disease)

0 = No 1 = Yes

☐

Comments:

c) Cerebrovascular Disease (e.g. hypertension, stroke)

0 = No 1 = Yes

☐

Comments:

d) Cardiovascular Disease (e.g. coronary/ischaemic heart disease)

0 = No 1 = Yes

☐

Comments:

e) Gynaecological Disease (e.g. polycystic ovarian syndrome,
endometriosis)

0 = No 1 = Yes

☐

Comments:

f) Disease of the Nervous System (e.g. epilepsy, Parkinson's, Alzheimer's)

0 = No 1 = Yes

☐

Comments:

g) Disease of Bones and Joints (e.g. osteoporosis, arthritis)

0 = No 1 = Yes

☐

Comments:

h) Disease of immune system and allergic disorder
(e.g. rheumatoid arthritis, rhinitis, type 1 diabetes, AIDS)

0 = No 1 = Yes

☐

Comments:

i) Muscle disease (e.g. chronic fatigue, myopathy)

0 = No 1 = Yes

☐

Comments:

j) Metabolic diseases (e.g. diabetes)

0 = No 1 = Yes

☐

Comments:

k) Other

0 = No 1 = Yes

☐

Comments:

2. Now I would like to ask you some questions about this pregnancy:

a. Are you aware of any problems with this pregnancy? 0 = No 1 = Yes ☐

If yes, please describe

b..Have you been seen by an obstetrician (pregnancy specialist doctor) during this pregnancy?

0 = No 1 = Yes ☐

If yes: Were you informed of any problems with this pregnancy? 0 = No 1 = Yes ☐

If yes, please describe

c. Have your pregnancy ultrasound scans been normal? 0 = No 1 = Yes ☐

If no: Have you been alerted to any particular problems, or had to have extra scans or investigations? 0 = No 1 = Yes ☐

If yes, please describe

d. Have you experienced any vaginal bleeding during this pregnancy? 0 = No 1 = Yes ☐

If yes: Please describe what happened when the bleeding was at its worst.

.....

Was it spotting, slight, moderate, or heavy? (If unsure, use usual bleeding during menstrual periods as a reference. Ask whether sanitary towels or tampons were used, and if so how many)

1 = Spotting 2 = Moderate 3 = Heavy N/A = -88 ☐

How long did the bleeding last for?

Did you get a scan, see a doctor or midwife about this, or were you admitted to hospital?

0 = None of these 1 = Scan 2 = Saw doctor 3 = Admitted ☐

3. Which hand do you normally use to write? 0 = R 1= LT 2 = Ambidextrous ☐

4. Are you HIV positive? 0 = No 1 = Yes ☐

5. Have you ever had a head injury? 0 = No 1 = Yes ☐

If yes, describe most recent event:

6. Have you ever had an MRI scan? 0 = No 1 = Yes 3= Unsure ☐

OBSTETRIC HISTORY

Could I ask some questions now about this pregnancy and your obstetric history?

1. Could you tell me the date of your last menstrual period?
2. What is your expected date of delivery according to your last menstrual period?
3. What is your expected date of delivery according to your ultrasound scan?
Researcher should verify by looking at ultrasound scan report in maternity notes.
4. Was your baby conceived through IVF, egg or sperm donation? 0 = No 1 = Yes
If yes: Was it: 1 = IVF 2 = egg donor 3 = sperm donor
5. How old were you when your menstrual periods began?
6. How many children do you have?
- 6a. If already has a child/children:
 Was your child [were any of your children] born at less than 37 weeks gestation?
If yes, how many? (0=none)
 At what gestation age(s) _____
7. Have you ever had any pregnancies that that did not result in a live birth, for example, a pregnancy that miscarried, was terminated, or ended in a stillbirth?
 0 = No 1 = Yes
If no, skip to Q.8
 - a) Spontaneous miscarriage at <16 weeks *If yes, how many? (0=none)*
 At what gestation age(s) _____ *(not for data entry)*
 - b) Elective termination of pregnancy *If yes, how many? (0=none)*
 At what gestation age(s) _____ *(not for data entry)*
 - c) An intrauterine death at 16 - <24 weeks *If yes, how many? (0=none)*
 - d) An intrauterine death at 24 - 28 weeks *If yes, how many? (0=none)*
 - e) An intrauterine death at 29 - 33 weeks *If yes, how many? (0=none)*

- f) An intrauterine death at >34 weeks If yes, how many? (0=none)
8. Can I just check, including this one, how many pregnancies have you had in total?
9. Have you had a Urinary Tract Infection in this pregnancy? 0 = No 1 = Yes
- 10a. What was your weight before you became pregnant? _____ stones & lbs **OR** _____ kg
 Convert to kgs for data entry (using project conversion tables)
- 10b. What is your height? _____ feet & inches **OR** _____ cms
 Convert to cms for data entry (using project conversion tables)
11. Are you planning a hospital or a home birth? 0 = Hospital 1 = Home birth

SMOKING HISTORY, TEA & COFFEE AND ALCOHOL CONSUMPTION

1. Have you ever smoked cigarettes or other tobacco products?
 0 = No 1= Yes IF NO, skip to question 11
2. Do you smoke at all nowadays?
 0 = No 1= Yes IF NO, skip to Q.8
3. What is the nicotine level of your usual brand of cigarettes?
 1 = 0.9 mg or less 2 = 1.0-1.2 mg 3 = 1.3 mg or more
 Usual brand (not for data entry): _____
4. About how many cigarettes a day do you usually smoke? _____
 (If smokes roll-ups, ask for best estimate)
5. Are you currently smoking more or less than before you became pregnant?
 1 – No change
 2 – Fewer cigarettes smoked now
 3 – More cigarettes smoked now
 IF NO CHANGE, skip to question 10
6. When in the pregnancy did you begin smoking more or less? Weeks: _____

7. On average, how many cigarettes were you smoking before the change? _____

Skip to question 11

8 When did you quit smoking? Date:

9 On average, how many cigarettes were you smoking before you quit? _____

10. Do you currently use nicotine replacements such as gum or patches?
0 = No 1 = Yes

IF YES, ask for details (gum/patches, strength, frequency): _____

11. Does anyone else living in your household smoke?
1 = No 1 = Partner only 2 = Others only 3 = Partner & others

If others: How many others?

12. Do you currently drink tea or coffee?
Coffee: 0 = No If yes, number of cups per week _____

Tea: 0 = No If yes, number of cups per week _____

13. How often do you currently have a drink containing alcohol?
0 = Never 3 = 2-3 times a week
1 = Monthly or less 4 = 4+ times a week
2 = 2-4 times a month

If never, skip to next section.

14. How many drinks containing alcohol do you have on a typical day when you are drinking?
1 = 1 or 2 2 = 3 or 4 3 = 5 or 6 4 = 7 to 9 5 = 10 or more

15. How many units of alcohol do you currently drink in a typical week? 0 = None

Appendix C Protocol for blood sample acquisition and processing

Venepuncture protocol for researchers for baseline:

Blood should be taken between 12pm and 3pm at baseline

1. Create a vacuum in the Monovette tubes by pulling out the syringe mechanism.
2. Record the time on the blood collection record.
3. Use the Greiner blood collection set with holder. Cannulate with the butterfly needle, secure the butterfly with Micropore tape.
4. Fill the blood collection tubes in the following order (be careful to ensure the needle goes into the Monovette tube):
 - i. Plain Monovette (white top)
 - ii. 2x EDTA Monovette (red top) (after blood collection gently invert the tube 4-5 times)
5. Release the tourniquet, remove the butterfly needle, use cotton wool and apply pressure to the venepuncture site, and then cover with a small plaster after haemostasis.
6. Label the blood tubes with participant ID no. and initials and the date.

After the venepuncture visit:

Blood should be processed within 2 hours of venepuncture

Record all the times as required on the blood collection record

Transport the Monovette samples to the laboratory allowing enough time so that they can be processed within 2 hours. Transport in accordance with the Standard operating procedure for blood transport (**appendix 10e**).

1. Transport the red top (EDTA) and the white top (Serum) Monovette tubes inside mini bio isotherm boxes according to the SOP (**appendix 10e**). Samples should be processed according to the protocols below.
2. Complete the blood collection record accurately with all times and details of any deviations from the protocol or unexpected occurrences.

Processing plasma and serum

1. Use the refrigerated centrifuge:
 - i. Pre-cool to 4 °C
 - ii. Set the speed at 1500G
 - iii. Set acceleration and brake at maximum
 - iv. Set the centrifuge timer for 10 minutes
2. Centrifuge the three Monovette tubes at 4 °C for 10 minutes.
3. Record the start-time of centrifugation.
4. Process the samples keeping the cryovials on ice.
5. Using an aseptic technique:
 - i. Transfer 11 x aliquots of plasma (from the red-top tube) and 6 x aliquots of serum (from the white-top tube) to cryovials.
 - ii. Freeze the samples immediately at -80 °C.

Appendix D Protocol for maternal saliva sample collection

Maternal saliva sample collection:

STUDY DOCUMENTS:

PRAM maternal saliva collection record and instructions version 5 0 3-12-11

The researcher should pre-prepare 6 **Salivettes, which must contain a polymer (not cotton) swab:**
Label the tubes with the barcode labels, press firmly and rub the labels carefully to ensure they stay fixed on.

The researcher should take time to explain the process carefully to the participant. Provide the participant with a mechanical timer to keep.

Sampling is done at awakening, and at +15, +30, and +60 minutes following awakening, and at midday and 8pm. The researcher should go over the requirements, documents and equipment in detail with participants and let them handle a demonstration example. It should be emphasised to participants that the timings and instructions are critical, so they should collect their samples exactly according to the instructions provided, but that it is important that we know what actually happened, so if they make an error, e.g. in times, eating or drinking, or in using the wrong tube, this should be recorded on the collection record.

Participants should be offered the option of having text reminders for sampling.

In circumstances when participants are adamant that they cannot follow the protocol, they should collect a minimum of awakening, +30, midday and 8pm samples. In circumstances when participants are adamant that they cannot use the polymer swabs, they can be offered Sorbette arrows as an alternative; these should be prepared then posted to the participant by the researcher.

Participants should store their samples in their domestic refrigerator (not freezer) and post them in a stamped addressed envelope on a Monday or Tuesday, or the researcher can visit the participant to collect the samples.

Researchers should know when to expect samples, and chase up those which do not arrive when expected.

On receipt of saliva samples at IoP, the researcher should check the samples, labels, and collection record form. Freeze samples immediately either in Perinatal freezer (-20) in the 6th floor phlebotomy room, Main Building, IoP, or in the designated -20 freezer at JBC.

For samples initially stored in the Perinatal freezer, transport the samples in a polystyrene box or cool box, with ice blocks or wet ice (the samples should not thaw) to the JBC within 1 week using the using the EU compliant tertiary bag. Place the samples in the designated freezer and update the appropriate sample tracking database with the new storage details.

Samples will be used to quantify levels of cortisol

Appendix E Maternal saliva collection record

MATERNAL SALIVA SAMPLE COLLECTION RECORD



P.R.A.M.
Psychiatry
Research and
Motherhood

Subject ID (no.-initials)

				-			
--	--	--	--	---	--	--	--

Researcher's Initials

--	--	--

Baseline

32 wks gestation

MRI

8 weeks postnatal

1 year postnatal

DATE OF COLLECTION (DD-MM-YY)

		-			-		
--	--	---	--	--	---	--	--

PLEASE USE THE TIMER - TO ENSURE YOU TAKE YOUR SAMPLES AT THE CORRECT TIMES

	Time	Complete
Awakening		BOX 1
Awakening +15 minutes		BOX 2
Awakening +30 minutes		BOX 3
Awakening +60 minutes		BOX 4
Midday		BOX 5
8pm		BOX 6

BOX 1

Wake up (before 10am)

Immediately after waking up collect your saliva as on the instruction diagram. Place the swab *under your tongue* and leave it there for 1-2 minutes, then place it as shown in the tube marked 0 min.

Then close the tube firmly and store in the fridge in the bag supplied.

Write here the EXACT TIME OF AWAKENING: _____

PLEASE NOW USE THE TIMER PROVIDED

Try to sit down and relax in the next hour. **YOU MUST NOT BRUSH YOUR TEETH AND MUST NOT HAVE ANYTHING TO EAT, DRINK OR SMOKE FOR THE NEXT HOUR.**

You may drink water if you need to, but only immediately AFTER you have taken a sample.

BOX 2

15 minutes after waking up, collect your saliva in the tube marked 15 min.

Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here: _____

BOX 3

30 minutes after waking up, collect your saliva in the tube marked 30 min.

Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here: _____

BOX 4

60 minutes after waking up, collect your saliva in the tube marked 60 min.

Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here: _____

*******YOU CAN NOW HAVE BREAKFAST AND BRUSH YOUR TEETH! *******

*******YOU SHOULD NOT EAT OR DRINK ANYTHING, SMOKE OR BRUSH YOU TEETH**

IN THE 30 MINUTES BEFORE NOON.*****

BOX 5

At 12 noon, before lunch, collect your saliva in the tube marked 12 pm.

Then close the tube firmly and store in the fridge in the bag supplied.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here: _____

*******YOU SHOULD NOT EAT OR DRINK ANYTHING, SMOKE OR BRUSH YOUR TEETH**

IN THE 30 MINUTES BEFORE 8PM.*****

BOX 6

At 8pm collect your saliva in the tube marked 8 pm.

Then close the tube firmly and store in the fridge in the bag supplied

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here: _____

Please note the name and time of any medication you have taken today (including the contraceptive pill):

Do you have any medical problems? If so, please list them here

Returning the samples:

Your researcher will tell you if they will collect your samples from you or if you should post them. Please remember to place all your samples in the fridge.

Posting: Please post your samples back to us the following Monday or Tuesday, and leave your samples in your fridge until posting.

Place all the samples in the plastic bag provided and seal it carefully. Place the bag and the collection record into the stamped addressed envelope provided. Please remember to make a note of the date you will post the specimens on the record form before sealing the envelope.

Date posted: __ / __ / __

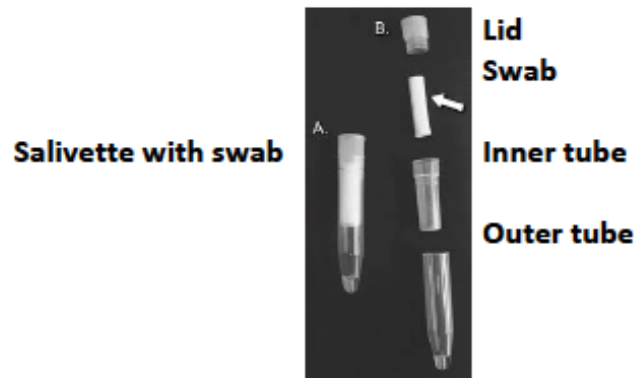
If you have any questions about the process, please call the research team on 020 7848 5009.

Office use only:

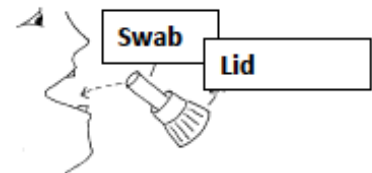
[Date of sample receipt: __ / __ / __

Date of sample storage: __ / __ / __]

How to collect the saliva samples:



1. Take care to find the salivette tube marked with the appropriate time.
2. Carefully remove the lid (the part on the end with ridges on).
3. Tip the swab into the lid and use this to place the swab under the front of your tongue. Do not touch the swab with your fingers.
4. Keep the swab in place for 1-2 minutes to ensure that it is saturated.
5. Take the swab out of your mouth with the help of the lid (so you are not touching the swab with your fingers).
6. Carefully tip the swab into the salivette tube without touching it with your fingers.
7. Replace the lid firmly.
8. Store the samples in your fridge.



THANK YOU.

Appendix F Protocol for 8 week postnatal interview

Subject ID (no.-initials) -

Researcher's Initials

DATE - -



Psychiatry Research & Motherhood (PRAM) Study

8 Week postnatal interview booklet

Baby (feeding, delivery, health & medication)

Medication record

Medical conditions

Smoking, tea & coffee consumption

Demographic changes since last interview

Data codes.

- 66 Participant didn't know
- 77 Participant refused to answer
- 88 Not applicable
- 99 Not assessed/missing

Note to researcher:

If participant's response does not correspond to a specific answer code, please write in the response for later coding.

**PLEASE REMEMBER TO CHECK PREVIOUS ASSESSMENTS
FOR ANY MISSING DATA THAT NEEDS TO BE CLARIFIED
AND NOTE BELOW**

**MISSING DATA FROM PREVIOUS ASSESSMENTS
QUESTIONS TO ASK AT 8 WEEK ASSESSMENT**

.....

.....

.....

.....

.....

.....

.....

.....

BACKGROUND DETAILS

I'd like to start by asking a few questions about you and your baby.

1. Have you had any problems with feeding [baby]? 0 = No 1 = Yes ☐

If yes, what problems?

2. How is [baby] being fed at present?

0 = Breast only 1 = Formula only 2 = Mixed breast and bottle ☐

- 2a. If currently formula, name of formula:

3. If formula only, did you breastfeed at all? 0 = No 1 = Yes ☐

If yes, for how long? days or weeks

Delivery

1. How long did you stay in hospital after your delivery? (days) Home delivery = -88 ☐

If more than 24 hours: Was there any reason that you or the baby needed to remain in hospital?

Details - mother:

.....

Details - baby:

.....

2. We'd like to ask about your experience of the delivery.

- a. On a scale of 0 to 10, how frightened were you during the birth? ☐

0 ("not frightened at all") to 10 ("extremely frightened").

- b. On a scale of 0 to 10, what was your overall experience of the birth? ☐

0 ("very good") to 10 ("extremely bad")

- c. On a scale of 1 to 4, to what degree did you feel taken care of during the birth? ☐

1 ("very good") to 4 ("very bad")

Baby's health

1. Has your baby needed to see a doctor at all since you left hospital?

0 = No 1 = GP only 2 = A&E visit 3 = Hospital admission 4 = Hospital OP ☐

If yes to any:

- 1.a. How many times has (s)he seen the GP? 0 = None ☐
For what reason(s)?
- 1.b. How many times has (s)he been to a hospital outpatients appointment? 0 = None ☐
For what reason(s)?
- 1.c. How many times has (s)he been to A&E? 0 = None ☐
For what reason(s)?
- 1.d. How many times has (s)he been admitted to hospital? 0 = None ☐
For what reason(s)?

Baby's current medication

1. Is your baby currently taking any regular medications, including oral medication (including dietary supplement), creams and inhalers? 0 = No 1 = Yes ☐
- a) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:
- b) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:
- c) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:
- d) Record any other drugs here in the same way
.....
2. Is your baby taking any as required medications, including oral medication (including dietary supplement), creams and inhalers? 0 = No 1 = Yes ☐
- a) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:
- b) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:
- c) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /

iii) Route of administration:

d) Record any others here in the same way

.....

Maternal current medication

1. Are you currently taking any regular medications, including oral medication (including dietary supplement), creams and inhalers?

0 = No 1 = Yes

☐

a) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

b) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

c) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

e) Record any other drugs here in the same way

.....

2. Are you currently taking any as required medications, including oral medication (including dietary supplement), creams and inhalers?

0 = No 1 = Yes

☐

a) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

b) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

c) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

d) Record any others here in the same way

.....

3. Did you take folic acid before or during this pregnancy?

0 = No 1 = Yes

☐

Start date (month / year)

End date (month / year)

MEDICAL HISTORY

I would like to ask you about any medical problems you might have currently:

Do you have any chronic medical conditions? 0 = No 1 = Yes ☐

Comments

I would like to go through a list with you to check if you have any of the following disorders?
Check individually through each system below:

a) Respiratory or lung disease (e.g. asthma, Chronic obstructive airways disease) 0 = No 1 = Yes ☐

Comments

b) Endocrine disorder (e.g. thyroid disease, Cushing's disease) 0 = No 1 = Yes ☐

Comments

c) Cerebrovascular Disease (e.g. hypertension, stroke) 0 = No 1 = Yes ☐

Comments

d) Cardiovascular Disease (e.g. coronary/ischaemic heart disease) 0 = No 1 = Yes ☐

Comments.....

e) Gynaecological Disease (e.g. polycystic ovarian syndrome, endometriosis) 0 = No 1 = Yes ☐

Comments

f) Disease of the Nervous System (e.g. epilepsy, Parkinson's, Alzheimer's) 0 = No 1 = Yes ☐

Comments

g) Disease of Bones and Joints (e.g. osteoporosis, arthritis) 0 = No 1 = Yes ☐

Comments

h) Disease of immune system and allergic disorder (e.g. rheumatoid arthritis, rhinitis, type 1 diabetes, AIDS) 0 = No 1 = Yes ☐

Comments

i) Muscle disease (e.g. chronic fatigue, myopathy) 0 = No 1 = Yes ☐

Comments

j) Metabolic diseases (e.g. diabetes) 0 = No 1 = Yes ☐

Comments

k) Other 0 = No 1 = Yes ☐

Comments

Have you experienced any physical problems related to the pregnancy or delivery?

0 = No 1 = Yes

☐

What was the problem?

I would like to go through a list with you to check if you have had any of the following problems since we saw you in pregnancy.

Check individually through each problem below:

Pregnancy conditions

1) Hypertension

0 = No 1 = Yes

☐

Details

2) Gestational diabetes

0 = No 1 = Yes

☐

Details

3) Nausea and vomiting or hyperemesis

0 = No 1 = Yes

☐

Details

4) Placental abruption

0 = No 1 = Yes

☐

Details

Major physical morbidities

1) Thromboembolism (VTE, DVT or PTE)

0 = No 1 = Yes

☐

(Venous thromboembolism (VTE) includes deep venous thrombosis (DVT) and pulmonary thromboembolism (PTE).

Details

2) Postpartum haemorrhage (PPH)

0 = No 1 = Yes

☐

Primary PPH (within the first 24 hours of the birth) or secondary PPH (after 24 hours & up to six weeks after the birth).

Details

3) Pre-eclampsia or eclampsia

0 = No 1 = Yes

☐

Details

4) Puerperal sepsis.

0 = No 1 = Yes

☐

Details

Common health problems

5) Excessive tiredness or fatigue

0 = No 1 = Yes

☐

6) Backaches

0 = No 1 = Yes

☐

- | | | |
|--|-------------------|--------------------------|
| 7) Sore or cracked nipples | 0 = No 1 = Yes | <input type="checkbox"/> |
| 8) Perineal pain | 0 = No 1 = Yes | <input type="checkbox"/> |
| 9) Haemorrhoids (piles) | 0 = No 1 = Yes | <input type="checkbox"/> |
| 10) Bowel problems (e.g. excessive or unusual diarrhoea or constipation) | 0 = No 1 = Yes | <input type="checkbox"/> |
| 11) Bad headaches | 0 = No 1 = Yes | <input type="checkbox"/> |
| 12) Bladder problems | 0 = No 1 = Yes | <input type="checkbox"/> |
| 13) Red or tender breasts or mastitis | 0 = No 1 = Yes | <input type="checkbox"/> |
| 14) Sexual problems | 0 = No 1 = Yes | <input type="checkbox"/> |
| 15) Anything else? | 0 = No 1 = Yes | <input type="checkbox"/> |

If yes, specify:

SMOKING, TEA & COFFEE AND ALCOHOL CONSUMPTION

1. Do you smoke at all nowadays? 0 = No 1 = Yes ☐
 IF NO, skip to Q.4

2. What is the nicotine level of your usual brand of cigarettes? ☐

1 = 0.9 mg or less 2 = 1.0-1.2 mg 3 = 1.3 mg or more

 Usual brand (not for data entry):

3. About how many cigarettes a day do you usually smoke? _____ ☐☐
 (If smokes roll-ups, ask for best estimate)

4. Do you currently use nicotine replacements such as gum or patches? 0 = No 1 = Yes ☐
 IF YES, ask for details (gum/patches, strength, frequency): _____
 _____ (not for data entry)

5. Does anyone else living in your household smoke? ☐

0 = No 1 = Partner only 2 = Others only 3 = Partner & others

If others: How many others? ☐☐

 IF NO, skip to Q.7

6. About how many cigarettes a day do other household members usually smoke? ☐☐

Partner: ☐☐

Others: ☐☐

7. Does anyone, including visitors, smoke in the flat/house?

0 = No, smoking not allowed anywhere in the home
1 = Yes, but only in certain rooms/places
2 = Yes, only if baby not in room
3 = Yes, anywhere

8. Do you ever take your baby to visit any places where other people smoke?
For example, someone else's house, or smoky public place?

0 = Never 1 = Sometimes 2 = Often

If sometimes or often, what places?

9. On average, how many hours a day would you say your baby spends in a place where people are smoking, both in and out of the flat/house?

10. Do you think that children's health is affected by people smoking in the home?

0 = a great deal 1 = quite a lot 2 = a little 3 = not at all

11. Do you currently drink tea or coffee?

Coffee: 0 = No If yes, number of cups per week _____

Tea: 0 = No If yes, number of cups per week _____

12. How often do you currently have a drink containing alcohol?

0 = Never 3 = 2-3 times a week
1 = Monthly or less 4 = 4+ times a week
2 = 2-4 times a month

If never, skip to next section.

13. How many drinks containing alcohol do you have on a typical day when you are drinking?

1 = 1 or 2 2 = 3 or 4 3 = 5 or 6 4 = 7 to 9 5 = 10 or more

14. How many units of alcohol do you currently drink in a typical week? 0 = None or <1

Demographic questions

1. Are you currently married or living with someone? *If no: Do you have a boyfriend or partner?*

1 Single (no partner) 3 Cohabiting
2 Single (with partner) 4 Married

If has partner, is this the same partner as you had at the first interview?

0= No

1 = Yes

☐

2. Are you working at the moment?

1 Employed, working

2 Employed, on maternity leave

3 Employed, on sick leave

4 Unemployed/ full-time mother

5 Student

6 Other

☐

Appendix G Protocol for 1 year postnatal interview

Subject ID (no.-initials) -

Researcher's Initials

DATE - -



Psychiatry Research & Motherhood (PRAM) Study

Chapter 1 1 year postnatal interview booklet

Baby (feeding, health & medication)

Maternal Medication record

Medical conditions & pregnancies

Smoking, tea & coffee consumption

Demographic changes since last interview

Data codes.

- 66 Participant didn't know
- 77 Participant refused to answer
- 88 Not applicable
- 99 Not assessed/missing

Note to researcher:

If participant's response does not correspond to a specific answer code, please write in the response for later coding.

**PLEASE REMEMBER TO CHECK PREVIOUS ASSESSMENTS FOR ANY MISSING DATA
THAT NEEDS TO BE CLARIFIED AND NOTED
MISSING DATA FROM PREVIOUS ASSESSMENTS**

QUESTIONS TO ASK AT 1 YEAR ASSESSMENT

.....

.....

.....

.....

.....

.....

.....

.....

DEMOGRAPHIC DETAILS

I'd like to start by asking a few questions about you and your baby.

1. What milk is [baby] having at present?

0 = Breast only 1 = Formula only 3. Cow's milk 4 = Mixed

☐

If mixed, details:

2. Did you breastfeed at all?

0 = No 1 = Yes

☐

2.a. If yes: How old was [baby] when breast-feeding stopped? (months) N/A = -88

3. How old was [baby] when (s)he started solids? (months) N/A = -88

Delivery

1. How long did you stay in hospital after your delivery? (days)

Home delivery = -88

If more than 24 hours: Was there any reason that you or the baby needed to remain in hospital?

Details - mother:

.....

Details - baby:

.....

Baby's health

I would like to ask you about any medical problems your baby may have currently:

1. Does (s)he have any chronic medical conditions?

0 = No 1 = Yes

☐

Details:

.....

2. Has (s)he had any serious illness?

0 = No 1 = Yes

☐

Details:

.....

3.. How many times has (s)he seen the GP (other than for routine check)? 0 = None

☐

For what reason(s)?

4.. How many times has (s)he been to a hospital outpatients appointment? 0 = None ☐
For what reason(s)?

5. How many times has (s)he been to A&E? 0 = None ☐
For what reason(s)?

6. How many times has (s)he been admitted to hospital? 0 = None ☐
For what reason(s)?

IF NOT KNOWN: Was (s)he conceived through IVF or donor egg or sperm? 0 = No 1 = Yes ☐

If yes, was it through: 1 = IVF 2 = Donor egg 3 = Donor sperm ☐

Baby's current medication

1. Is your baby currently taking any regular medications, including oral medication (including dietary supplement), creams and inhalers? 0 = No 1 = Yes ☐

a) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

b) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

c) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

d) Record any other drugs here in the same way
.....

2. Is your baby taking any as required medications, including oral medication (including dietary supplement), creams and inhalers? 0 = No 1 = Yes ☐

a) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

b) Drug Name:
i) Dose (mg/day)
ii) Start date (month / year) /
iii) Route of administration:

- c) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 d) Record any others here in the same way

Maternal current medication

1. Are you currently taking any regular medications, including oral medication (including dietary supplement), creams and inhalers? 0 = No 1 = Yes ☐

- a) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 b) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 c) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 e) Record any other drugs here in the same way

2. Are you currently taking any as required medications, including oral medication (including dietary supplement), creams and inhalers? 0 = No 1 = Yes ☐

- a) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 b) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 c) Drug Name:
 i) Dose (mg/day)
 ii) Start date (month / year) /
 iii) Route of administration:
 d) Record any others here in the same way

MEDICAL HISTORY

I would like to ask you about any medical problems you might have currently:

Do you have any chronic medical conditions? 0 = No 1 = Yes ☐

Comments

I would like to go through a list with you to check if you have any of the following disorders?

Check individually through each system below:

a) Respiratory or lung disease (e.g. asthma, Chronic obstructive airways disease) 0 = No 1 = Yes ☐

Comments

b) Endocrine disorder (e.g. thyroid disease, Cushing's disease) 0 = No 1 = Yes ☐

Comments

c) Cerebrovascular Disease (e.g. hypertension, stroke) 0 = No 1 = Yes ☐

Comments

d) Cardiovascular Disease (e.g. coronary/ischaemic heart disease) 0 = No 1 = Yes ☐

Comments

e) Gynaecological Disease (e.g. polycystic ovarian syndrome, endometriosis) 0 = No 1 = Yes ☐

Comments

f) Disease of the Nervous System (e.g. epilepsy, Parkinson's, Alzheimer's) 0 = No 1 = Yes ☐

Comments

g) Disease of Bones and Joints (e.g. osteoporosis, arthritis) 0 = No 1 = Yes ☐

Comments

h) Disease of immune system and allergic disorder (e.g. rheumatoid arthritis, rhinitis, type 1 diabetes, AIDS) 0 = No 1 = Yes ☐

Comments

i) Muscle disease (e.g. chronic fatigue, myopathy) 0 = No 1 = Yes ☐

Comments

j) Metabolic diseases (e.g. diabetes) 0 = No 1 = Yes ☐

Comments

k) Other 0 = No 1 = Yes ☐

Comments

PREGNANCIES SINCE INDEX BABY

Are you currently pregnant? 0 = No 1 = Yes ☐

If yes, how many weeks pregnant are you?

Since [your baby] was born, have you had any more pregnancies
for example, ending in a miscarriage? 0 = No 1 = Yes ☐

If yes, was the outcome:

a) Spontaneous miscarriage at <16 weeks (0=none) ☐

At what gestation age _____ (not for data entry)

b) Elective termination of pregnancy (0=none) ☐

At what gestation age _____ (not for data entry)

c) An intrauterine death at 16 - <24 weeks (0=none) ☐

d) An intrauterine death at 24 - 28 weeks (0=none) ☐

e) An intrauterine death at 29 - 33 weeks (0=none) ☐

f) An intrauterine death at >34 weeks (0=none) ☐

SMOKING, TEA & COFFEE AND ALCOHOL CONSUMPTION

1. Do you smoke at all nowadays? 0 = No 1 = Yes ☐

IF NO, skip to Q.4

2. What is the nicotine level of your usual brand of cigarettes? ☐

1 = 0.9 mg or less 2 = 1.0-1.2 mg 3 = 1.3 mg or more

Usual brand (not for data entry): _____

3. About how many cigarettes a day do you usually smoke? _____ ☐

(If smokes roll-ups, ask for best estimate)

4. Do you currently use nicotine replacements such as gum or patches? 0 = No 1 = Yes ☐

IF YES, ask for details (gum/patches, strength, frequency): _____

_____ (not for data entry)

5. Does anyone else living in your household smoke?

0 = No 1 = Partner only 2 = Others only 3 = Partner & others

If others: How many others?

IF NO, skip to Q.7

6. About how many cigarettes a day do other household members usually smoke?

Partner:

Others:

7. Does anyone, including visitors, smoke in the flat/house?

0 = No, smoking not allowed anywhere in the home

1 = Yes, but only in certain rooms/places

2 = Yes, only if baby not in room

3 = Yes, anywhere

8. Do you ever take your baby to visit any places where other people smoke?

For example, someone else's house, or smoky public place?

0 = Never

1 = Sometimes

2 = Often

If sometimes or often, what places?

9. On average, how many hours a day would you say your baby spends in a place where people are smoking, both in and out of the flat/house?

TEA, COFFE & ALCOHOL

11. Do you currently drink tea or coffee?

Coffee: 0 = No If yes, number of cups per week _____

Tea: 0 = No If yes, number of cups per week _____

12. How often do you currently have a drink containing alcohol?

0 = Never

1 = Monthly or less

2 = 2-4 times a month

3 = 2-3 times a week

4 = 4+ times a week

If never, skip to next section.

13. How many drinks containing alcohol do you have on a typical day when you are drinking?

1 = 1 or 2 2 = 3 or 4 3 = 5 or 6 4 = 7 to 9 5 = 10 or more

14. How many units of alcohol do you currently drink in a typical week?

0 = None or <1

Demographic questions

1. Are you currently married or living with someone? *If no: Do you have a boyfriend or partner?*

- 1 Single (no partner)
- 2 Single (with partner)

- 3 Cohabiting
- 4 Married

☐

If has partner, is this the same partner as you had at the first interview?

0= No

1 = Yes

☐

2. Are you working at the moment?

- 1 Employed, working
- 2 Employed, on maternity leave
- 3 Employed, on sick leave

- 4 Unemployed/ full-time mother
- 5 Student
- 6 Other

☐

If employed, ask:

How old was [baby] when you went back to work?

(months)

Who looks after [baby] when you're at work?

- 1 Child's father/ mother's partner
- 2 Child's grandparent
- 3 Other adult relative

- 4 Childminder
- 5 Nursery
- 6 Other or combination (*specify*)

☐

.....

Appendix H Protocol for infant saliva collection

Infant saliva sample at 8 Weeks and 1 Year Postnatal:

STUDY DOCUMENTS:

PRAM infant saliva collection record and instructions for 8 wks and 1 year postnatal version 6.1 29-3-12

The researcher should pre-prepare 4 Salivettes with barcode labels. Discard the polymer swab and keep the inner plastic part of the Salivette. Prepare 4 Salimetrics Childrens Swabs and some clean gloves in a specimen bag to take to the assessment.

The researcher should take time to explain the process carefully to the participant. The researcher will obtain (or show the mother how to obtain) a saliva sample from the infant before and exactly 20 minutes after the immunization, and show the mother how to obtain the samples on the 2nd day at awakening and 8pm.

The researcher should go over the requirements, documents and equipment in detail with participants and let them handle a demonstration example. It should be emphasised to participants that the timings and instructions are critical, so they should collect their samples exactly according to the instructions provided, but that it is important that we know what actually happened, so if they make an error, e.g. in times, or in using the wrong tube, this should be recorded on the collection record.

Participants should be offered the option of having text reminders for sampling.

If a researcher attends the immunizations they should bring those samples back to IoP that day with the appropriate page of the collection record (first page) and the instructions for the researcher (last page). Participants should store their samples in their domestic refrigerator (not freezer) and keep them until collection by the researcher.

On receipt of saliva samples at IoP, the researcher should check the samples, labels, and collection record form. Freeze samples immediately either in Perinatal freezer (-20) in the 6th floor phlebotomy room, Main Building, IoP, or in the designated -20 freezer at JBC.

For samples initially stored in Perinatal freezer, transport the samples in a cool box, with ice blocks or wet ice using the EU compliant tertiary bag. Place the sample in the designated freezer in JBC within 1 week, and update the appropriate sample tracking database with the new storage details.

Samples will be used to quantify levels of cortisol.

Appendix I Infant saliva collection record



P.R.A.M.
Psychiatry
Research and
Motherhood

BABY SALIVA SAMPLE COLLECTION RECORD

Subject ID (no.-initials) **b** -

Researcher's Initials

8 weeks postnatal 1 year postnatal (please delete)

DAY 1 (to be completed by researcher and brought back to the IOP with the saliva samples)

DATE OF COLLECTION (DD-MM-YY) - -

N.B. Ideally the samples should not be taken for at least 15 minutes after a feed.

What time was baby's most recent nap? Start: _____ Finish: _____

What time was baby's most recent feed? Start: _____ Finish: _____

Before the immunization collect baby's saliva with the help of the researcher (as on the instructions for the researcher). Take the tube marked "PRE." Place the Salimetrics children's swab *under child's tongue* and leave it there for 60 to 90 seconds (the swab can be placed under the tongue for a few seconds at a time and reintroduced as needed), then place the wet end in the salivette tube, the researcher will fold the swab and close the tube firmly.

EXACT TIME OF SAMPLING: _____

TIME OF Immunization: _____

Comments e.g. Was mother present? Did baby cry much?

Exactly 20 minutes after the immunization collect baby's saliva in the tube marked "POST" as for the 1st sample. Then close the tube firmly and store in the bag supplied.

- What time is it now? _____
- Was baby fed? (Y/N) If yes, when and for how long: Start: _____ Finish: _____
- Did baby fall asleep? (Y/N) If yes, when: Start: _____ Finish: _____

Please note the name and time of any medication baby is taking

Does baby have any medical problems? If so, please list them here

Office use only: [Date of sample receipt: __ / __ / __ Date of sample storage: __ / __ / __]

PRAM infant saliva collection record and instructions for 8 weeks and 12 months postnatal.
Version 7.1 24 July 2013

BABY SALIVA SAMPLE COLLECTION RECORD DAY 2

P.R.A.M.
Psychiatry
Research and
Motherhood

Subject ID (no.-initials) **b** -

Researcher's Initials

DATE OF COLLECTION (DD-MM-YY) - -

8 weeks postnatal

1 year postnatal (please delete)

N.B. Ideally the samples should not be taken for at least 15 minutes after a feed.

Immediately after your baby wakes up and before feeding collect your baby's saliva using the arrows as on the instruction diagram. Take the tube marked "AM." Place the Sorbette arrows *under your baby's tongue* and leave them there for a total of 60-90 seconds (the Sorbette can be placed under the tongue for 15 to 30 seconds at a time and reintroduced as needed), then place them back in the tube, close the tube firmly and store in the bag supplied.

EXACT TIME OF SAMPLING: _____

What time did your baby wake up? _____

What time was your baby's most recent feed? Start: _____ Finish: _____

At 8pm collect your baby's saliva using the arrows in the tube marked "8 PM".

Then close the tube firmly and **store in the fridge** in the bag supplied

- What time is it now? _____
- What time was the baby's most recent nap? Start: _____ Finish: _____
- What time was the baby's most recent feed? Start: _____ Finish: _____

Please note the name and time of any medication your baby is taking

Does your baby have any medical problems? If so, please list them here

Your researcher will tell you if they will collect your samples or will ask you to post them.

Posting the samples:

Please post your samples back to us the following Monday or Tuesday, and leave your samples in your fridge until posting. Place all the samples in the plastic bag provided and seal it carefully. Place the bag and the collection record into the stamped addressed envelope provided. Please remember to make a note of the date you will post the specimens on the record form before sealing the envelope.

Date posted: __ / __ / __

If you have any questions about the process, please call the research team on 020 7848 5009

Office use only: [Date of sample receipt: __ / __ / __ Date of sample storage: __ / __ / __]

PRAM infant saliva collection record and instructions for 8 weeks and 12 months postnatal.
Version 7.1 24 July 2013

p 2 of 3

How to collect the saliva samples using Salimetrics children's swab

Salivette*



Salimetrics children's swab:



1. Please wash and dry your hands carefully or wear gloves.
2. Take care to find the tube (salivette) marked with the appropriate time.
3. Take the swab, avoid touching the swab with your fingers where possible and carefully follow the instructions and the diagram.
4. Securely hold one end of the swab and place the other end under the child's tongue. Reintroduce the swab as necessary for a total of 60-90 seconds.
5. Place the saturated swab directly into the salivette, by folding, as shown in the diagram on the right, or by cutting it with clean scissors (the scissors should be cleaned with an alcohol swab):
6. Place the samples in a fridge until collection by a researcher.

N.B. The researcher will take the samples from the immunisation directly to the lab and place them in a freezer.



Appendix J Standard operating procedure for CRH magnetic RIA

CORTICOTROPIN RELEASING FACTOR (CRF, CRH)

EXPLANATION OF THE TEST

Corticotropin-releasing hormone (CRH), originally named corticotropin-releasing factor (CRF), and also called corticoliberin, is a polypeptide hormone and neurotransmitter involved in the stress response.

Corticotropin-releasing hormone (CRH) is a 41-amino acid peptide derived from a 191-amino acid preprohormone. CRH is secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress. Marked reduction in CRH has been observed in association with Alzheimer's disease, and autosomal recessive hypothalamic corticotrophin deficiency has multiple and potentially-fatal metabolic consequences including hypoglycemia and hepatitis. In addition to being produced in the hypothalamus, CRH is also synthesized in peripheral tissues, such as T lymphocytes, and is highly expressed in the placenta. In the placenta, CRH is a marker that determines the length of gestation and the timing of parturition and delivery. A rapid increase in circulating levels of CRH occurs at the onset of parturition, suggesting that, in addition to its metabolic functions, CRH may act as a trigger for parturition.

CRH is produced by neuroendocrine cells in the paraventricular nucleus of the hypothalamus and is released from neurosecretory terminals of these neurons into the primary capillary plexus of the hypothalamo-hypophyseal portal system. The portal system carries the CRH to the anterior lobe of the pituitary, where it stimulates corticotropes to secrete corticotropin (ACTH) and other biologically-active substances (for example β -endorphin). α -helical CRH-(9–41) acts as a CRH antagonist.

METHOD

CRF magnetic RIA kit is supplied by Phoenix Pharmaceuticals, Inc. 330 Beach Road, Burlingame, California 94010.

The assay is based upon the competition of 125 I-peptide and unlabeled peptide (standard or unknown) binding to the limited quantity of antibodies specific for peptide in each reaction mixture. The assay utilises anti-peptide magnetic beads to separate bound and unbound peptide.

As the quantity of standard or unknown sample in the reaction increases, the amount of 125 I-peptide able to bind to the antibody is decreased. By measuring the amount of 125 I-peptide bound as a function of the concentration of peptide (in standard reaction mixtures), it is possible to construct a standard curve from which the concentration of peptide in the unknown sample can be determined.

TECHNICAL DATA

Intra-assay precision

	Level 1	Level 2	Level 3	Level 4
--	---------	---------	---------	---------

N	6	6	6	6
Mean (pg/mL)	279	185	21	15
CV%	3.0	8.8	7.3	6.7

Inter-assay precision

	Level 1	Level 2	Level 3	Level 4
N	6	6	6	6
Mean (pg/mL)	166	134	50	26
CV%	11.8	2.3	3.3	6.1

Sample requirements

Patient should be fasting 10 - 12 hours and should not be on any corticosteroid, ACTH, or estrogen medications, if possible, for at least 48 hours prior to collection of specimen. An am specimen is preferred.

The specimen should be plasma from an EDTA vacutainer. The usual precautions for venipuncture should be observed.

Blood samples should be spun at 4°C and serum/plasma aliquoted into a 2mL tube and placed in the freezer at -70°C until assayed. Plasma is stable for up to twelve months when stored at -70°C. Avoid repeat freeze-thaw cycles.

To assay the sample in duplicate a minimum of 40 µL is required. However, the sample tube should contain at least a volume of 100 µL to account for the dead volume and dilution.

Linearity

Up to 1280 pg/mL, any results above this concentration are reported as >1280 pg/mL. The samples if necessary can be diluted using RIA Buffer and repeated in the next batch.

Sensitivity

The minimal detectable CRF concentration is 5.0 pg/mL.

Appendix K Standard operating procedure for CRHBP ELISA



Standard Operating Procedure 26

DEPARTMENT
OF WOMEN'S
AND CHILDREN'S
HEALTH



STANDARD OPERATING PROCEDURE

Number	026	Version & Issue	1.3
Title	CRHBP ELISA		
Author	Jo Drury	Date	25/04/08
Approved	Dr S Quenby	Date	25/04/08
Review Date	November 2014	Last reviewed	25/10/13

Background

Human Corticotrophin Releasing Hormone Binding Protein (CRHBP) is a 37 kDa secreted glycoprotein and an important molecule to study in relation to Corticotrophin Releasing Hormone (CRH) as it is involved in regulating available CRH by forming complexes with CRH to form a circulating 41 kDa complex. This is important in preterm labour as it is thought that CRH may act as a "biological clock" determining the length of human pregnancy such that levels increase prior to delivery due to decreased CRHBP binding.

Definition

This SOP will explain how to assay CRHBP using sandwich ELISA methodology including the use of the plate reader.

Health and Safety Precautions

The main health and safety risks to this procedure arise from the use of sulphuric acid, sodium azide and biological samples. Sulphuric acid causes severe burns on contact and damages mucous membranes on inhalation. Sodium azide is an irritant to the respiratory tract and mucous membranes. It is highly toxic on ingestion and by contact. Laboratory coat, protective gloves and safety glasses should be worn. When removing plasma samples from the -80°C freezer, cryogenic gloves should be worn. Plasma should be added to and decanted from the plate in the Category 2 Hood (Lab 5, room 1124).

EQUIPMENT INFORMATION

Plate reader: Multiskan Ascent (Thermo Electron Corporation) connected to Dell PC running Thermo Ascent software.

Technical specialist: Paul Carson

Unit 5, The Ringway Centre
Edison Road
Basingstoke
Hampshire, RG21 6YH
0870 6099023
07970 099147 (mobile)
0870 6099202 (fax)
Paul.carson@thermofisher.com
www.thermo.com

Plate shaker/incubator: Dynatech Varishaker~Incubator

Microtiter plates (Corning, 3369)

Plate sealers (Anachem 100-SEAL-PLT)

Other useful SOPs associated with 026

Standard Operating Procedure 26

SOP 28: ELISA plate reader use.

Procedure:

Solutions required:

PBS

40 g NaCl (BDH)

1g KCl (BDH)

7.5 g Na₂HPO₄·2H₂O (BDH)

1 g KH₂PO₄ (BDH)

Add 1 l dH₂O, mix to dissolve and adjust pH to 7.4 with 2M NaOH if necessary.

Make up to 5 l with dH₂O.

TBS

60 g Tris (Sigma)

87 g NaCl (BDH)

Add 1 l dH₂O, mix to dissolve and adjust pH to 7.6 with conc HCl.

Make up to 10 l with dH₂O. Use within 2-4 weeks.

Wash buffer

PBS/0.05% Tween 20 (BDH) (2 ml 50% Tween 20 + 2 l PBS)

Blocking buffer

PBS/1% BSA/5% sucrose/0.05% sodium azide (499 ml PBS, 5 g BSA, 25 g sucrose, 1250 µl 20% sodium azide)

MAB2796 CRHBP monoclonal antibody (R&D systems)

Reconstitute the antibody in 0.2 ml sterile PBS (producing 500µg/ml stock).

E0431 biotinylated swine anti-rabbit polyclonal antibody (DAKO)

Standard diluent

PBS/0.1% BSA (50 µl 10% BSA/5 ml PBS)

Diluent for polyclonal rabbit-anti-human CRHBP antibody RJW, detection antibody

(biotinylated swine anti-rabbit polyclonal (DAKO) and Strp/HRP (R&D Systems):

TBS/0.05% Tween 20/0.1% BSA (50 µl 50% Tween 20, 500 µl 10% BSA, 50 ml TBS)

300 nmol/l CRHBP fragment (E Linton, Oxford; stored -80°C: freezer 3, shelf 2, lab 2 (room 1127))

TMB/H₂O₂ substrate solution (Sigma)

Stop solution (1M H₂SO₄) (BDH)

Plasma analysis Plate 1:

1. Prepare primary antibody: 80µl anti-CRH-BP monoclonal antibody + 9920µl PBS → 4 µg/ml
2. Coat plate with 100 µl/well primary antibody and cover with plate sealer.
3. Incubate room temperature overnight.
4. Remove blocking buffer from fridge to warm to room temperature.
5. Decant onto paper towels and blot vigorously. Dispose of waste towels in clinical waste bin.

Standard Operating Procedure 26

6. Using a wash bottle filled with wash buffer, fill the wells forcefully, decant the wash solution into the sink, blot onto paper towels and repeat 2x to wash a total of 3x with wash buffer.
7. Block with 300 µl blocking buffer, incubate 1 h, 37°C (Dynatech Varishaker~Incubator, incubator setting 3 ¼, shaker setting 3 ½).
8. Remove plasma samples from freezer 3, room 1127. Once defrosted, samples must be kept on ice/cold packs at all times.
9. Prepare CRHBP standards in PBS/0.1% BSA by serial doubling dilutions of 300 nmol/l stock:

[CRHBP] (pmol/l)	300 nmol/l CRHBP std	PBS/0.1% BSA (columns 1&2)
2000	4 µl	596µl
1000	300 µl	300 µl
500	300 µl	300 µl
250	300 µl	300 µl
125	300 µl	300 µl
62.5	300 µl	300 µl
31.25	300 µl	300 µl

10. Decant and wash 3x with wash buffer (as described in step 6)
11. Pipette 100 µl standards, sample or diluent according to the plate diagram below:

	1,2	3,4	5,6	7,8	9,10	11,12
A	Diluent (PBS /0.1% BSA)					
B	31.25 pmol/l CRHBP					
C	62.5 pmol/l CRHBP					
D	125 pmol/l CRHBP					
E	250 pmol/l CRHBP					
F	500 pmol/l CRHBP					
G	1000 pmol/l CRHBP					
H	2000 pmol/l CRHBP					Anon A VP

12. Cover and incubate with shaking 1 h, 37°C.
13. Prepare 1:200 anti-CRHBP antibody RJW 'bleed 1': 60 µl/11940 µl diluent).
14. Decant and wash 3x with wash buffer
15. Add 100 µl/well anti-CRHBP antibody RJW.
16. Cover and incubate 1h, 37°C, shaking.
17. Prepare detection antibody stock solution 1:100 (10 µl + 990 µl diluent)
 - 1:5000, 240 µl 1:100 stock + 11760 µl diluent
18. Decant and wash 3x with wash buffer
19. Add 100 µl/well detection antibody
20. Cover and incubate 30 minutes, room temperature, shaking.
21. Turn on Dell PC and Multiskan Ascent plate reader. See "SOP 28, ELISA plate reader use" for more details.
22. Enter the appropriate sample identification information onto the plate plan.
23. Save the file under a new name.

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THIS IS A CONTROLLED DOCUMENT. DO NOT COPY

Standard Operating Procedure 26

24. Prepare 1:200 Strp/HRP solution: 60 µl/11940 µl diluent.
25. Decant and wash plate 3x with wash buffer.
26. Add 100 µl Strp/HRP/well.
27. Cover and incubate 30 min, room temperature.
28. Remove TMB/substrate solution from fridge to warm to room temperature.
29. Decant and wash plate 3x with wash buffer.
30. Add 100 µl substrate TMB/H₂O₂/well.
31. Incubate 2-5 min, dark, room temperature.
32. Add 50 µl stop solution.
33. Place the plate (with no cover) on the plate reader.
34. Read OD₄₅₀ and OD₅₄₀ by clicking "Start" from the main menu of the Ascent software.
35. Click on the results tab to access the results data.
36. A standard curve is prepared by clicking on the graph icon from the main menu. The fit type should be "linear". Results are calculated automatically by the software. Ensure that all results fall within the linear portion of the standard curve.
37. Enter results onto appropriate spreadsheet/database.

SOP History

Original SOP prepared by J Drury 14th April 2008.

Updated 25th April 2008.

Updated 25th October 2013 by J Drury to correct omissions/errors and change logos.

Appendices -Associated Documents

This will have a list of associated template letters and guidelines relevant to the SOP.

	Document	Location
1	COSHH assessment of ELISA assays	COSHH Assessment folder in room 0607
2	Risk Assessment of "Separation of plasma components from human whole blood and analysis of plasma proteins using ELISA"	Risk Assessment folder in room 0607
3		
4		
5		
6		
7		
8		
9		

Appendix L Standard operating procedure for serum cortisol magnetic ELISA

CORTISOL

EXPLANATION OF THE TEST

Cortisol is the major glucocorticoid secreted by the human adrenal cortex gland. Glucocorticoids are synthesised in response to the release of adrenocorticotrophic hormone (ACTH) from the anterior lobe of the pituitary gland. Pituitary ACTH is in turn regulated by corticotrophin releasing factor (CRF) secreted by the hypothalamus. CRF release is stimulated in response to physical and psychological stress.

The actions of cortisol form a vital part of the response to stress, particularly:

- to act as an anti-inflammatory agent;
- to maintain blood pressure;
- to promote the synthesis of carbohydrates (gluconeogenesis) from skeletal muscle protein.

Hypothalamic CRF release is inhibited by cortisol, thus forming a negative feedback loop. Under normal circumstances, cortisol secretion exhibits a diurnal variation, with cortisol levels being highest at around 9 o'clock in the morning and lowest at around midnight.

Serum cortisol is usually measured in one of three ways:

- in a single sample (random, 9am or midnight);
- as part of a stimulation test;
- as part of a suppression test.

Stimulation tests (e.g. the synacthen test which uses synthetic ACTH) are employed to demonstrate the secretory capacity of the adrenal gland and thus to exclude deficiency states (e.g. Addison's disease).

Suppression tests (e.g. the dexamethasone suppression test) are used to examine normal feedback and to exclude the presence of hypersecretion syndromes such as Cushing's Syndrome.

METHOD

Cortisol reagent is supplied by Siemens Healthcare Diagnostics Ltd, Newton House, Sir William Siemens Square, Frimley, Camberley, Surrey, GU16 8QD

Sample is incubated with cortisol labelled with acridinium ester (AE) (*'Lite' reagent*) and an anti-cortisol antibody which is covalently bound to paramagnetic particles (PMP) (*solid-phase reagent*). Labelled cortisol competes with cortisol in the patient sample for the limited binding sites on the PMP-bound antibody. After the incubation period a magnetic field is applied to the reaction mixture causing the solid-phase PMP (which contains the bound labelled cortisol) to be held at the side of the reaction cuvette while the liquid phase is aspirated. The cuvette contents are washed with deionised water which is then aspirated, again with the magnetic field applied. Acid reagent (containing hydrogen peroxide) is then added to the cuvette to begin the light-emission reaction with the acridinium ester. The cuvette is then moved to the luminometer at which point base reagent is added to enhance the light reaction. The

light intensity is measured immediately and converted to 'relative light units' (RLU). The RLU value has an inverse relationship with cortisol concentration (i.e. as more labelled cortisol is bound less patient cortisol will be bound). The RLU value is compared with those of the analyte master curve to obtain the analyte concentration via the relevant curve-fit algorithm.

TECHNICAL DATA

Intra-assay precision

	Level 1	Level 2	Level 3	Level 4	Level 5
Mean (nmol/L)	107.05	155.33	390.95	759.55	1024.97
CV%	3.69	3.09	2.89	3.82	2.98

Inter-assay precision

	Level 1	Level 2	Level 3	Level 4	Level 5
Mean (nmol/L)	107.05	155.33	390.95	759.55	1024.97
CV%	6.58	4.92	4.22	4.25	4.98

Sample requirements

Serum or heparinised plasma may be used. Specimens stored overnight should be refrigerated. Gel separation tubes may be used. The Centaur uses 20 µL of sample in the test, although the minimum volume required in the specimen is 300 µL (Vacutainers). Small samples should be transferred to 13 mm inserts placed inside a 13 mm vacutainer or carrier tube- where the dead volume is reduced to 25 µL.

Sensitivity

The minimum detectable concentration is 30 nmol/L.

Linearity

The assay is linear up to concentrations of 1800 nmol/L.

Limitations

Circulating cortisol results from patients receiving Prednisolone or Prednisone (which is converted to Prednisolone in vivo) therapy may be falsely elevated. Exercise caution with cortisol determinations for patients undergoing therapy with these and structurally related synthetic corticosteroids.

Standardization

The ADVIA Centaur Cortisol assay is standardized using internal standards manufactured analytically which are traceable to gas chromatography-mass spectroscopy (GCMS). The following equation describes the relationship between the cortisol standards and GCMS analysis throughout the range of the assay.

$$\text{ADVIA Centaur Cortisol} = 0.99 (\text{GCMS}) + 0.75 \mu\text{g/dL}, r = 0.99$$

Appendix M Standard operating procedure for saliva cortisol
ELISA



High Sensitivity

SALIVARY CORTISOL

ENZYME IMMUNOASSAY KIT

For Research Use Only

Item No. 1-3002, (Single) 96-Well Kit;

1-3002-5, (5-Pack) 480 Wells

Rev. August 2012

HS SALIVARY CORTISOL EIA KIT

Intended Use

The Salimetrics™ cortisol kit is a competitive immunoassay specifically designed and validated for the quantitative measurement of salivary cortisol. It is intended only for research use in humans and some animals.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.

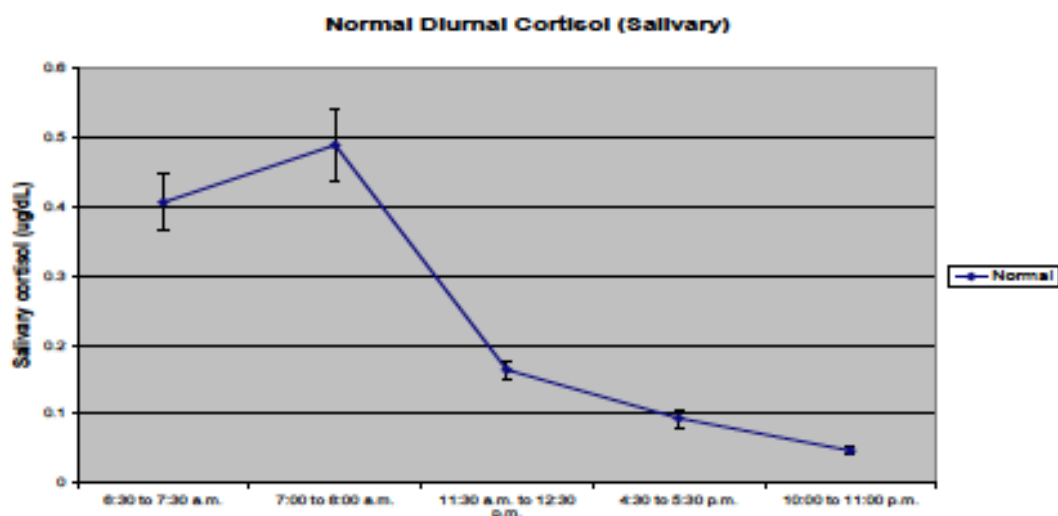
For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Cortisol (hydrocortisone, Compound F) is the major glucocorticoid produced in the adrenal cortex. (1) Cortisol production has a circadian rhythm, (2,3) with levels peaking in the early morning and dropping to lowest values at night. (4,5) Levels rise independently of circadian rhythm in response to stress. (6)

In blood, only about 5-10% of cortisol is in its unbound or biologically active form. The remaining cortisol is bound to serum proteins. (7) Unbound serum cortisol enters saliva via intracellular mechanisms; in saliva, the majority of cortisol remains unbound to protein. Salivary cortisol levels are unaffected by salivary flow rate and are relatively resistant to degradation from enzymes or freeze-thaw cycles. (8,9)

Studies consistently report high correlations between serum and salivary cortisol, indicating that salivary cortisol levels reliably estimate serum cortisol levels. (10-12)



(Internal Salimetrics Data, n=26. Time of cortisol peak will vary in individuals relative to their normal wake-up time.)

Test Principle

A microtitre plate is coated with monoclonal antibodies to cortisol. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, as measured by the intensity of color, is inversely proportional to the amount of cortisol present. (13)

Appendix N Correlations tables for results – Antenatal

Table 48: Plasma CRH and blood sampling details

	1	2	3	4	5	n
1 CRH	-					76
2 CRHBP	-.07	-				38
3 Gestational age at sample	.31**	.06	-			76
4 Time of sample acquisition	-.15	.19	-.21	-		76
5 Interval between sample acquisition and sample processing	.10	.06	.17	-.05	-	75
6 Interval between collecting sample and analysing CRHBP	-.16	.18	-.19	.40***	-.13	76

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. **p < .01, ***p < .001

Table 49: Plasma CRH and socio-demographic characteristics of the sample at baseline

	1	2	3	4	5	6	7	8	9	n
1 CRH	-									76
2 Maternal age at baseline	-.13	-								76
3 Maternal ethnicity white	.06	.48***	-							76
4 Level of qualification ≥ A levels	-.17	.49***	-.35**	-						76
5 Not working outside the home	.01	-.34**	.56***	.36**	-					76
6 Maternal professional or managerial	-.10	.47***	-.50***	-.60***	-.47***	-				74
7 Married	-.07	.44***	-.59***	-.40***	-.48***	-.47***	-			76
8 Paternal status unemployed	.06	-.04	-.10	-.07	-.12	.19	-.09	-		37
9 Paternal professional or managerial	-.43*	.02	-.35*	-.26	-.26	-.38*	-.16	.16	-	31
10 Paternal ethnicity white	-.02	.48**	-.72***	-.53***	-.50**	-.65***	-.57***	.23	-.31	38

Note. Pearson's *r* coefficients are presented for continuous variables. Point-biserial correlation coefficients are reported for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. **p* < .05, ***p* < .01, ****p* < .001.

Table 50: Plasma CRH and obstetric history and risk factors at baseline

	1	2	3	4	5	6	7	8	9	n
1 CRH	-									76
2 History of preterm birth	-.07	-								76
3 History of miscarriage	.00	.03	-							76
4 History of termination of pregnancy	.00	.11	-.03	-						76
5 History of intra-uterine death	.02	-.03	-.06	-.07	-					75
6 Parous	-.04	.21	.11	.11	.10	-				76
7 Age at menarche	-.11	-.14	.22	.09	-.01	-.06	-			40
8 Gestational age at baseline	.40***	.05	-.04	.17	.12	-.14	.14	-		76
9 Vaginal bleeding in index pregnancy	-.07	.30**	.13	-.07	.21	.10	.09	.03	-	75
10 UTI in index pregnancy	-.17	-.11	-.03	.01	-.06	-.22	.05	-.19	-.17	74

Note. Pearson's r coefficients are presented for continuous variables. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. Point-biserial correlation coefficients (based on ranked scores) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. ** $p < .01$, *** $p < .001$.

Table 51: Plasma CRH and medication, physical health and health behaviours at baseline

	1	2	3	4	5	6	7	8	n
1 CRH	-								76
2 Taking regular medication at baseline	-.18	-							76
3 Taking regular or PRN steroids at baseline	-.27	.80***	-						45
4 Chronic medical problem at baseline	.06	.24*	.50***	-					76
5 Pre-pregnancy BMI	-.10	-.25*	-.31	.01	-				72
6 Coffee use at baseline	.05	.06	.01	-.02	-.18	-			75
7 Tea use at baseline	.10	-.01	.03	.09	.09	-.12	-		75
8 Alcohol >2units/week at baseline	.13	-.03	-	-.07	-.07	.22	-.14	-	72
9 Smoking in pregnancy prior to baseline	.14	-.10	-.16	.07	.31**	-.12	.06	.17	72

Note. Spearman's correlation coefficients are presented for continuous variables. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p < .05, **p < .01, ***p < .001.

Table 52: Serum cortisol and blood sampling details

	1	2	3	n
1 Serum cortisol	-			42
2 Gestational age at sample	-.32*	-		42
3 Time of sample acquisition	-.01	-.04	-	42
4 Interval between sample acquisition and sample processing	-.08	.15	-.16	41

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. *p < .05

Table 53: Awakening cortisol and saliva sampling details at baseline

	1	2	3	4	5	n
1 Awakening cortisol	-					39
2 Gestational age at date of sample	.11	-				38
3 Time of awakening	-.16	-.01	-			39
4 Time of awakening sample	-.13	-.01	1.0***	-		39
5 Interval between awakening and sampling	.35*	-.07	.03	.08	-	39
6 Interval between sampling and freezing	.05	-.01	-.16	-.15	.16	36

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis; Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. * $p < .05$, *** $p < .001$.

Table 54: Evening cortisol and saliva sampling details at baseline

	1	2	3	n
1 Evening saliva cortisol	-			38
2 Gestational age at date of sample	-.20	-		38
3 Evening sample time	-.01	-.16	-	38
4 Interval between sampling and freezing	.08	.01	.19	36

Note. Spearman's correlation coefficients are presented

Table 55: Diurnal cortisol secretion and saliva sampling details at baseline

	1	2	3	4	n
1 Diurnal cortisol secretion	-				35
2 Gestational age at sample	-.05	-			32
3 Time of awakening	-.39*	-.09	-		35
4 Interval between awakening and sampling	.29	-.04	.02	-	35
5 Interval between sampling and freezing	.20	.00	-.16	.19	33

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. * $p < .05$

Table 56: CAR and saliva sampling details at baseline

	1	2	3	4	n
1 CAR (AUCg)	-				21
2 CAR (AUCi)	.24	-			21
3 Gestational age at sample acquisition	-.00	.27	-		20
4 Time of awakening	-.54*	-.27	-.18	-	21
5 Interval between sampling and freezing	.32	.08	-.09	-.60**	19

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis; Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. * $p < .05$, ** $p < .01$.

Table 57: Awakening saliva cortisol and saliva sampling details at 32 weeks gestation

	1	2	3	4	5	n
1 Awakening cortisol	-					66
2 Gestational age at date of sample	.10	-				65
3 Time of awakening	-.04	-.02	-			66
4 Time of awakening sample	-.06	-.01	.98***	-		66
5 Interval between awakening and sampling	-.10	-.30*	-.03	.16	-	66
6 Interval between sampling and freezing	-.15	-.17	-.11	-.04	.13	60

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis, *p <.05, ***<.001

Table 58: Awakening cortisol at 32 weeks gestation and socio-demographic characteristics of the sample at baseline

	1	2	3	4	5	6	7	8	9	n
1 Awakening cortisol	-									66
2 Maternal age at baseline	.28*	-								66
3 Maternal ethnicity white	.28*	.54***	-							66
4 Level of qualification ≥ A levels	.10	-.50***	.47***	-						66
5 Not working outside the home	-.08	-.32**	-.48**	-.42***	-					66
6 Maternal professional or managerial	.16	.50***	.57***	.63***	-.44**	-				66
7 Married	.18	.44***	.53***	.52***	-.32**	.51***	-			66
8 Paternal status unemployed	-.13	-.05	.09	.08	-.11	-.22	.09	-		35
9 Paternal professional or managerial	.11	.15	.41*	.23	-.22	.42*	.23	-.17	-	29
10 Paternal ethnicity white	.22	.58***	.82***	.63***	-.51**	.72***	.63***	-.26	.40*	35

Note. Pearson's r coefficients are presented for continuous variables. Point-biserial correlation coefficients are reported for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 59: Awakening cortisol at 32 weeks gestation and obstetric history and risk factors

	1	2	3	4	5	6	7	n
1 Awakening cortisol	-							66
2 History of preterm delivery	.16	-						66
3 History of miscarriage	.24	.09	-					66
4 History of termination of pregnancy	.05	.04	-.10	-				66
5 History of intrauterine death	.04	-.03	-.06	-.07	-			65
6 Parous	.08	.21	.14	.02	.12	-		66
7 Vaginal bleeding in index pregnancy	.29*	.40**	.12	.09	.23	.16	-	66
8 UTI in index pregnancy	-.18	-.12	.02	.08	-.07	-.26*	.05	65

Note. Point-biserial correlation coefficients are reported for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$, ** $p < .01$.

Table 60: Awakening cortisol at 32 weeks gestation, medication, physical health and health behaviours

	1	2	3	4	5	6	7	8	n
1 Awakening cortisol	-								66
2 Taking antidepressant at 32 weeks gestation	.04	-							64
3 Taking other regular medication at 32 weeks gestation	.01	.22	-						65
4 Taking regular or PRN steroids at 32 weeks gestation	.13	-.06	.35**	-					65
5 Chronic medical problem at baseline	.10	-.10	.20	.68***	-				66
6 Pre-pregnancy BMI	-.13	-.21	.06	-.05	-.01	-			62
7 Coffee use at baseline	.08	-.03	-.07	-.06	-.03	.19	-		66
8 Tea use at baseline	.08	-.17	.17	.31*	.14	.21	.11	-	66
9 Smoking in pregnancy prior to baseline	.10	.08	.08	.11	.00	.27*	.00	.19	62

Note. Spearman's correlation coefficients are presented for continuous variables. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p < .05, **p < .01, ***p < .001.

Table 61: Evening saliva cortisol and saliva sampling details at 32 weeks gestation

	1	2	3	n
1 Evening cortisol	-			61
2 Gestational age at date of sample	.43**	-		60
3 Time of evening sample	.14	.04	-	61
4 Interval between sampling and freezing	-.17	-.16	.10	56

Note. Spearman's correlation coefficients are presented, **p < .01.

Table 62: Evening saliva cortisol at 32 weeks gestation and baseline socio-demographic characteristics of the sample

	1	2	3	4	5	6	7	8	9	n
1 Evening cortisol	-									61
2 Maternal age at baseline	.04	-								61
3 Maternal ethnicity white	-.20	.49***	-							61
4 Level of qualification ≥ A levels	-.18	.44***	.30*	-						61
5 Not working outside the home	.29*	-.26*	-.52***	-.24	-					61
6 Maternal professional or managerial	-.12	.39**	.44***	.59***	-.34**	-				60
7 Married	-.18	.34**	.60***	.44***	-.43**	.44***	-			61
8 Paternal status unemployed	.01	-.06	-.10	-.07	-.12	-.22	-.10	-		34
9 Paternal professional or managerial	-.34	.15	.32	.19	-.19	.33	.15	-.18	-	29
10 Paternal ethnicity white	-.08	.45**	.74***	.54**	-.43*	.71***	.67***	-.26	.29	34

Note. Pearson's correlation coefficients are presented for continuous variables. Point-biserial correlation coefficients are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p < .05, **p < .01, ***p < .001.

Table 63: Evening cortisol at 32 weeks gestation, obstetric history and obstetric risk factors

	1	2	3	4	5	6	7	n
1 Evening cortisol	-							61
2 History of preterm delivery	.07	-						61
3 History of miscarriage	.35**	-.11	-					61
4 History of termination of pregnancy	.20	.08	-.07	-				61
5 History of intrauterine death	-.02	-.02	-.07	-.08	-			60
6 Parous	-.03	.19	.13	.01	.13	-		61
7 Vaginal bleeding in index pregnancy	.14	.31*	.05	.10	.22	.16	-	61
8 UTI in index pregnancy	.01	-.10	.02	.10	-.06	-.19	.05	61

Note. Point-biserial correlation coefficients are reported for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$, ** $p < .01$.

Table 64: Evening saliva cortisol, medications and physical health at 32 weeks gestation

	1	2	3	4	5	6	7	8	9	n
1 Evening cortisol	-									61
2 Taking antidepressant at 32 weeks gestation	-.06	-								59
3 Taking other regular medication at 32 weeks gestation	-.12	.21	-							61
4 Taking regular or PRN steroids at 32 weeks gestation	-.07	-.06	.40**	-						61
5 Taking any regular medication or PRN steroids	-.04	.55***	.72***	.64***	-					61
6 Chronic medical problem at baseline	-.03	-.10	.19	.60***	.32*	-				61
7 Pre-pregnancy BMI	.09	-.23	.03	-.06	-.09	.06	-			58
8 Coffee use at baseline	.24	-.03	-.05	-.04	-.01	-.02	-.21	-		61
9 Tea use at baseline	.17	-.19	.17	.37**	.19	.16	.11	-.10	-	61
10 Smoking in pregnancy prior to baseline	.22	-.09	.04	.07	-.05	.08	.24	-.04	-.02	59

Note. Spearman's correlation coefficients are presented for continuous variables. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 65: Diurnal cortisol secretion (AUC) and saliva sampling details at 32 weeks gestation

	1	2	3	4	n
1 Diurnal cortisol (AUC am to pm)	-				53
2 Gestational age at sample	.33*	-			52
3 Time of awakening	-.03	.10	-		53
4 Interval between awakening and evening samples	.25	.11	-.85***	-	53
5 Interval between sampling and freezing	-.13	-.20	-.14	.05	49

Note. Spearman's correlation coefficients are presented. *p < .05, ***p , .001.

Table 66: Diurnal cortisol secretion (AUC) at 32 weeks gestation and baseline socio-demographic characteristics of the sample

	1	2	3	4	5	6	7	8	9	n
1 Diurnal cortisol (AUC am to pm)	-									53
2 Maternal age at baseline	.23	-								53
3 Maternal ethnicity white	.21	.58***	-							53
4 Level of qualification ≥ A levels	-.04	.47***	.39**	-						53
5 Not working outside the home	-.01	-.28*	-.40**	-.27*	-					53
6 Maternal professional or managerial	.14	.46***	.49***	.61***	-.32*	-				53
7 Married	.05	.38**	.54***	.51***	-.30*	.49***	-			53
8 Paternal status unemployed	-.09	-.07	.08	.08	-.11	-.24	.09	-		30
9 Paternal professional or managerial	-.02	.08	.36	.20	-.14	.39	.20	-.20	-	25
10 Paternal ethnicity white	.25	.60**	.76***	.58**	-.36	.65***	.75***	-.30	.35	29

Note. Pearson's correlation coefficients are presented for continuous variables. Point-biserial correlation coefficients are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p < .05, **p < .01, ***p < .001.

Table 67: Diurnal cortisol secretion (AUC) at 32 weeks gestation and obstetric history and obstetric risk factors

	1	2	3	4	5	6	7	n
1 Diurnal cortisol (AUC am to pm)	-							53
2 History of preterm delivery	.23	-						53
3 History of miscarriage	.23	-.09	-					53
4 History of termination of pregnancy	-.01	.13	-.24	-				53
5 History of intrauterine death	-.00	-.03	-.06	-.07	-			52
6 Parous	.14	.20	.16	-.08	.14	-		53
7 Vaginal bleeding in index pregnancy	.34*	.33*	.07	.19	.23	.18	-	53
8 UTI in index pregnancy	-.22	-.11	.00	.14	-.07	-.17	.08	53

Note. Point-biserial correlation coefficients are reported for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$.

Table 68: Diurnal cortisol secretion (AUC) at 32 weeks gestation medication and physical health

	1	2	3	4	5	6	7	8	9	n
1 Diurnal cortisol (AUC am to pm)	-									53
2 Taking antidepressant at 32 weeks gestation	.02	-								53
3 Taking any regular medication at 32 weeks gestation	.02	.43*	-							53
4 Taking regular or PRN steroids at 32 weeks gestation	.20	-.07	.35*	-						53
5 Taking any regular medication or PRN steroids	.14	-.54***	.79***	.63***	-					53
6 Chronic medical problem at baseline	.14	-.11	.16	.63***	.33*	-				53
7 Pre-pregnancy BMI	-.11	.18	.09	.04	.07	.04	-			50
8 Coffee use at baseline	.30*	.04	.17	.02	.02	.01	-.17	-		53
9 Tea use at baseline	.22	.18	.23	.39**	.18	.17	.15	-.10	-	53
10 Smoking in pregnancy prior to baseline	.06	-.09	.06	.12	-.01	.01	.35*	.06	.01	51

Note. Spearman's correlation coefficients are presented for continuous variables. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 69: CAR and saliva sampling details at 32 weeks gestation

		1	2	3	4	n
1	CAR (AUCg)	-				28
2	CAR (AUCi)	.66***	-			28
3	Gestational age at sampling	-.15	-.26	-		27
4	Time of awakening	-.12	-.31	.07	-	28
5	Interval between sampling and freezing	.31	.61**	-.28	-.18	24

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. **p <.01, ***p <.001.

Table 70: CAR at 32 weeks gestation and baseline socio-demographic characteristics of the sample

	1	2	3	4	5	6	7	8	9	n
1 CAR (AUCi)	-									28
2 Maternal age at baseline	.32	-								28
3 Maternal ethnicity white	.24	.53**	-							28
4 Level of qualification \geq A levels	.19	.21	.51**	-						28
5 Not working outside the home	.25	-.25	-.48*	-.28	-					28
6 Maternal professional or managerial	.30	.17	.43*	.63***	-.12	-				28
7 Married	.25	.26	.44*	.44*	-.20	.34	-			28
8 Paternal status unemployed	-.24	-.11	.08	.08	-.13	-.28	.08	-		26
9 Paternal professional or managerial	.03	.20	.33	.33	-.09	.44*	.33	-.18	-	25
10 Paternal ethnicity white	.27	.43*	.81***	.59**	-.49**	.60**	.50**	-.38	.44*	28

Note. Pearson's r coefficients are presented for continuous variables. Point-biserial correlation coefficients are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. * $p < .05$, ** $p < .01$, *** $p < .001$.

Table 71: CAR at 32 weeks gestation, obstetric history and obstetric risk factors

	1	2	3	4	5	6	7	n
1 CAR (AUCi)	-							28
2 History of preterm delivery	.10	-						28
3 History of miscarriage	-.02	.19	-					28
4 History of termination of pregnancy	-.14	-.13	-.24	-				28
5 History of intrauterine death	.20	.05	.10	-.09	-			28
6 Parous	.31	.32	.25	-.03	.22	-		28
7 Vaginal bleeding in index pregnancy	.03	.37	-.03	.24	.26	.26	-	28
8 UTI in index pregnancy	-.31	-.16	.10	-.05	-.11	-.17	.09	28

Note. Point-biserial correlation coefficients are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables.

Table 72: CAR, medication and physical health at 32 weeks gestation

	1	2	3	4	5	6	7	8	9	n
1 CAR (AUCi)	-									28
2 Taking antidepressant at 32 weeks gestation	-.12	-								27
3 Taking other regular medication at 32 weeks gestation	-.15	.41	-							27
4 Taking regular or PRN steroids at 32 weeks gestation	-.05	-.06	-.59**	-						27
5 Taking any regular medication or PRN steroids	-.16	.41*	.16	.59**	-					27
6 Chronic medical problem at baseline	-.03	-.06	.14	1.0***	.59**	-				28
7 Pre-pregnancy BMI	-.29	-	.10	.04	.10	.04	-			27
8 Coffee use at baseline	-.01	.17	.15	.04	.15	.03	.05	-		28
9 Tea use at baseline	.03	.23	.26	.46*	.26	.42*	.19	.11	-	28
10 Smoking in pregnancy prior to baseline	-.27	-.08	.07	.28	.07	.23	.51**	.01	.18	28

Note. Spearman's correlation coefficients are presented. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p <.05*, **p <.01, ***p <.001.

Appendix O Correlations tables for results – Obstetric outcomes

Table 73: Gestational age at birth and baseline socio-demographic factors

	1	2	3	4	5	6	7	8	9	n
1 Gestational age at birth	-									58
2 Maternal age at baseline	.26*	-								58
3 Maternal ethnicity white	.30*	.41**	-							58
4 Level of qualification ≥ A levels	.13	.43**	.27*	-						58
5 Not working outside the home	-.26*	-.33**	-.57***	-.38**	-					58
6 Maternal professional or managerial	.19	.50***	.46***	.59***	-.45***	-				56
7 Married	.29*	.39**	.56***	.35**	-.50***	.46***	-			58
8 Paternal ethnicity white	.08	.47*	.67***	.54**	-.59**	.73***	.59**	-		29
9 Paternal status unemployed	.22	-.04	.13	.10	-.16	-.22	.15	-.25	-	26
10 Paternal professional or managerial	.24	.31	.30	.23	-.32	.39	.17	.24	-.18	22

Note. Spearman's correlation coefficients are presented. Point-biserial correlation coefficients (based on ranked scores) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p <.05, **p <.01, ***p <.001.

Table 74: Gestational age at birth and obstetric history and obstetric risk factors

	1	2	3	4	5	6	7	8	9	n
1 Gestational age at birth	-									58
2 History of preterm birth	-.17	-								58
3 History of miscarriage	-.19	-.09	-							58
4 History of termination of pregnancy	.02	.28*	-.04	-						58
5 History of intra-uterine death	.12	-.03	-.06	-.09	-					58
6 Parity	-.16	.19	.24	.34*	.04	-				58
7 Age at menarche	.04	-.20	.28	.09	-.03	.09	-			31
8 Gestational age at baseline	.07	-.01	-.21	.26*	.13	.03	.08	-		58
9 Vaginal bleeding in index pregnancy	-.12	.15	-.01	.15	.27*	.11	-.10	-.08	-	57
10 UTI in index pregnancy	.07	-.09	.00	-.02	-.06	-.16	-.15	-.16	.12	56

Note. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. Point-biserial correlation coefficients (based on ranked scores) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p < .05.

Table 75: Gestational age at birth and antenatal physical health

	1	2	3	4	5	6	7	8	9	10	11	n
1 Gestational age at birth	-											58
2 Chronic medical problem at baseline	.03	-										58
3 Taking regular medication at baseline	-.22	.13	-									58
4 Taking regular or PRN steroids at baseline	-.04	.85***	.21	-								34
5 Chronic medical problem at 32 weeks gestation	.03	.92***	.11	.75***	-							58
6 Taking antidepressant at 32 weeks gestation	-.28*	-.08	.24	-.06	-.09	-						50
7 Taking other regular medication at 32 weeks gestation	-.16	-.07	.32*	-.09	.22	.38**	-					55
8 Taking regular or PRN steroids at 32 weeks gestation	-.04	.58***	.19	.85***	.73***	-.08	.32*	-				55
9 Pre-pregnancy BMI	.27*	.01	-.16	-.08	.02	-.24	.18	.05	-			54
10 Coffee use at baseline	.19	-.10	.03	.04	-.06	-.06	-.03	.09	-.15	-		57
11 Tea use at baseline	.17	.15	-.18	.21	.23	-.18	.05	.29*	.19	-.03	-	57
12 Any smoking in the index pregnancy	-.05	.05	-.13	-.12	.18	-.11	.16	.08	.27	.03	-.02	55

Note. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables. *p < .05, **p < .01, ***p < .001.

Appendix P Correlations tables for results – 8 weeks postnatal

Table 76: Infant cortisol and saliva sampling details at immunizations at 8 weeks postnatal

	1	2	3	4	5	6	n
1 Cortisol pre-immunization	-						54
2 Cortisol post-immunization	.15	-					54
3 Delta cortisol	-.26	.85***	-				54
4 Baby's age at immunization	.17	-.18	-.22	-			54
5 Pre-immunisation sample time	-.08	-.08	-.13	.18	-		54
6 Immunisation time	-.10	-.08	-.11	.20	.94***	-	54
7 Interval between 'pre' sample and immunization	.12	-.06	-.04	-.03	-.11	.14	54

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. ***p < .001.

Table 77: Infant cortisol at immunizations at 8 weeks postnatal and potential confounding factors

	1	2	3	4	5	6	7	8	n
1 Cortisol before the immunization	-								54
2 Cortisol after the immunization	.15	-							54
3 Delta cortisol	-.26	.85***	-						54
4 Infant taking regular medication	.11	.08	.06	-					54
5 Exposure to smoking <i>in utero</i>	-.02	.07	.09	.54**	-				53
6 Exposure to Sx of anxiety <i>in utero</i>	.24	.12	.20	.12	.40**	-			50
7 Mother on regular medication at 8 wk PN	.25	.12	.08	.35**	.23	.48***	-		53
8 Mother smoking at 8 wk PN	-.05	-.17	.05	.52**	.88***	.46*	.24	-	29
9 Duration of MDD in the postnatal period	.26	.21	.06	.03	-.08	.20	.48***	.59***	53

Note. Pearson's *r* coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. Phi coefficients are presented for associations between two dichotomous variables **p* < .05, ***p* < .01, ****p* < .001.

Sx = symptoms, 8 wk PN = 8 weeks postnatal

Table 78: Infant awakening cortisol and saliva sampling details at 8 weeks postnatal

	1	2	3	4	5	n
1 Awakening cortisol	-					61
2 Awakening time	-.08	-				56
3 Time of sample acquisition	-.01	.91***	-			60
4 Interval between awakening and sample acquisition	.06	-.32*	.02	-		56
5 Interval between end of feed and sample acquisition	-.17	.14	-.18	-.57***	-	36
6 Interval between sample acquisition and storage	-.18	-.17	-.23	-.09	-.08	51

Note. Spearman's correlation coefficients are presented. *p < .05, ***p < .001.

Table 79: Evening saliva cortisol and saliva sampling details at 8 weeks postnatal

	1	2	3	4	5	n
1 Evening cortisol	-					61
2 Time of sample acquisition	-.06	-				59
3 Interval between end of feed and sample acquisition	.09	.32	-			35
4 Interval between end of nap and sample acquisition	.09	.75***	.23	-		35
5 Duration of the nap prior to sample acquisition	.34	.19	.49**	.26	-	31
6 Interval between sample acquisition and storage	-.01	-.02	.13	.25	.21	50

Note. Spearman's correlation coefficients are presented. **p < .01, ***p < .001.

Appendix Q Correlations tables for results – 1 year postnatal

Table 80: Infant cortisol response to pain and saliva sampling details at 1 year postnatal

	1	2	3	4	5	6	n
1 Cortisol pre-immunization	-						43
2 Cortisol post-immunization	.53***	-					43
3 Delta cortisol	-.38*	.46**	-				43
4 Baby's age at immunization	.27	.13	-.05	-			43
5 Pre-immunisation sample time	-.22	-.06	.05	-.06	-		43
6 Immunisation time	-.20	-.11	.00	-.10	.91***	-	43
7 Interval between 'pre' sample and immunization	-.02	-.02	-.02	.21	.22	.43**	43

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. *p < .05, **p < .01, ***p < .001

Table 81: Infant cortisol at immunizations at 1 year postnatal and potential confounding factors

	1	2	3	4	5	6	n
1 Cortisol before the immunization	-						43
2 Cortisol after the immunization	.53***	-					43
3 Delta cortisol	-.38*	.46**	-				43
4 Exposure to Sx of anxiety <i>in utero</i>	.06	.23	.23	-			41
5 Duration of MDD between assessments	-.03	.09	.21	.46**	-		41
6 Mother on regular medication at 1 year PN	-.15	-.06	-.20	.33*	.38*	-	41
7 Mother awakening cortisol at 1 year PN	.17	-.05	-.17	-.19	.06	-.04	38

Note. Pearson's r coefficients are presented for continuous variables that permitted parametric analysis. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. *p < .05, **p < .01, ***p < .001.

Sx = symptoms, 1 year PN = 1 year postnatal

Table 82: Infant awakening cortisol and saliva sampling details at 1 year postnatal

	1	2	3	4	5	n
1 Awakening cortisol	-					53
2 Awakening time	-.10	-				50
3 Time of sample acquisition	-.14	.88***	-			50
4 Interval between awakening and sample acquisition	.07	-.19	.16	-		48
5 Interval between end of feed and sample acquisition	-.27	.23	-.18	-.54*	-	21
6 Interval between sample acquisition and storage	.10	.09	.09	.12	.14	34

Note. Spearman's correlation coefficients are presented. *p < .05, ***p < .001

Table 83: Infant evening cortisol and saliva sampling details at 1 year postnatal

	1	2	3	4	5	n
1 Evening cortisol	-					53
2 Time of sample acquisition	.04	-				49
3 Interval between end of feed and sample acquisition	-.37	.07	-			26
4 Interval between end of nap and sample acquisition	.20	-.36*	.16	-		30
5 Duration of the nap prior to sample acquisition	.15	-.35	.13	.37	-	28
6 Interval between sample acquisition and storage	.10	.18	.16	.11	.19	34

Note. Spearman's correlation coefficients are presented for continuous variables that did not permit parametric analysis. *p < .05

Table 84: Infant basal cortisol at 1 year postnatal and potential confounding factors

	1	2	3	4	5	6	n
1 Infant awakening cortisol	-						53
2 Infant evening cortisol	.11	-					53
3 Exposure to Sx of anxiety <i>in utero</i>	-.04	.23	-				50
4 Duration of MDD between assessments	.02	.26	.53***	-			52
5 Maternal BDI at 1 year postnatal	.04	.23	.50***	.31*	-		53
6 Mother on regular medication at 1 year postnatal	-.09	-.14	.12	.38**	.29*	-	51
7 Mother awakening cortisol at 1 year postnatal	.24	.10	-.12	-.02	.03	-.10	50

Note. Spearman's correlation coefficients are presented. Point-biserial correlation coefficients (based on ranked scores for continuous variables not permitting parametric analysis) are presented for associations between a continuous and a dichotomous variable. **p < .01, ***p < .001.

Sx = symptoms, BDI = Beck Depression Inventory

